PB90-145723

Interim Methods for Development of Inhalation Reference Doses

(U.S.) Environmental Protection Agency, Research Triangle Park, NC

Aug 89

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO.
EPA 600/8-88/066F

2.

3. RECIPIENT'S ACCESSION NO.
PB90 145723/AS

4. TITLE AND SUBTITLE
Interim Methods for Development of Inhalation Reference Doses

5. REPORT DATE
April 1989

6. PERFORMING ORGANIZATION CODE
600/23

7. AUTHOR(S)
Annie Jarabek, et al. (see list of authors)

8. PERFORMING ORGANIZATION REPORT NO.
ECAO-R-0204

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Environmental Criteria and Assessment Office (MD-52)
Office of Health and Environmental Assessment, ORD
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

10. PROGRAM ELEMENT NO.
A101

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Office of Health and Environmental Assessment (RD-689)
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC 20460

13. TYPE OF REPORT AND PERIOD COVERED
Methodology

14. SPONSORING AGENCY CODE
600/21

15. SUPPLEMENTARY NOTES

16. ABSTRACT

The document, developed by scientists in Office of Health and Environmental Assessment and Office of Health Research of the Office of Research and Development, describes methodology for the derivation of Inhalation Reference Doses (RfD_i's). An RfD_i is defined as an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous lifetime inhalation exposure to the human population (including sensitive subpopulations) that is likely to be without appreciable risk of adverse noncancer health effects. A detailed discussion of factors that determine inhaled dose, including respiratory anatomy and physiology and the physicochemical properties of the inhaled pollutant, is provided. Discussions of these areas include currently available information on interspecies variability. Issues related to the evaluation of study design and the generation and characterization of inhalation exposures are also discussed. Dosimetric adjustments and approaches for estimating human equivalent concentrations, which are used to operationally derive RfD_i's when extrapolating risk from animal exposure scenarios, are described for respiratory and extrarespiratory effects of both particulate and gaseous agents. Guidance on use of human data, choice of toxicity studies, application of uncertainty factors and the minimum data criteria for RfD_i development is also provided.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
reference dose risk assessment inhalation toxicology noncancer health effect respiratory system dosimetry extrapolation		

18. DISTRIBUTION STATEMENT Release Unlimited	**19. SECURITY CLASS** *(This Report)* Unclassified	**21. NO. OF PAGES** 213
	20. SECURITY CLASS *(This page)* Unclassified	**22. PRICE**

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

United States
Environmental Protection
Agency

Office of Health and
Environmental Assessment
Washington DC 20460

EPA 600 8-88 066F
August 1989

Research and Development

PB90-145723

EPA

Interim Methods for Development of Inhalation Reference Doses

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL INFORMATION SERVICE
SPRINGFIELD, VA. 22161

EPA/600/8-88/066F
August 1989

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Environmental Criteria and Assessment Offices
Office of Health and Environmental Assessment
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

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CONTENTS

Page

LIST OF TABLES vii
LIST OF FIGURES viii
LIST OF ABBREVIATIONS x
AUTHORS CONTRIBUTORS, AND REVIEWERS xii
GLOSSARY xv

1. INTRODUCTION 1-1

    1.1 DEVELOPING BENCHMARK VALUES IN THE U.S. EPA 1-1
    1.2 GENERAL PRINCIPLES OF NONCANCER TOXICITY RISK ASSESSMENT .. 1-3
    1.3 STATE-OF-THE-ART APPLICATIONS TO THE DEVELOPMENT OF THE INHALATION RfD METHODOLOGY 1-6

2. CONCEPTUAL BASIS FOR INHALATION RISK ASSESSMENT METHODOLOGY 2-1

    2.1 FACTORS CONTROLLING COMPARATIVE INHALED DOSE 2-1
        2.1.1 Respiratory Anatomy and Physiology 2-3
            2.1.1.1 Respiratory Regions and Branching Patterns 2-3
                2.1.1.1.1 Effect on aerosol deposition mechanisms 2-4
                2.1.1.1.2 Effect on gas deposition and uptake 2-6
            2.1.1.2 Clearance Mechanisms and Cell Types 2-12
            2.1.1.3 Summary 2-17
        2.1.2 Physicochemical Characteristics of the Inhaled Agent 2-17
            2.1.2.1 Particles 2-17
            2.1.2.2 Gases and Vapors 2-19
        2.1.3 Impact of Experimental Protocol 2-22
            2.1.3.1 Pharmacologic Effects of Agents 2-23
            2.1.3.2 Measurement Techniques 2-23
                2.1.3.2.1 Anesthesia 2-23
                2.1.3.2.2 Breathing pattern 2-24
                2.1.3.2.3 Equipment specifications 2-25
            2.1.3.3 Definitions/Underlying Assumptions 2-25
            2.1.3.4 Exposure Technology 2-25
                2.1.3.4.1 Inhalation modes 2-25
                2.1.3.4.2 Generation and characterization 2-27
                2.1.3.4.3 Exposure regimen 2-28
        2.1.4 Summary 2-30
    2.2 PORTAL-OF-ENTRY CONSIDERATIONS: ASPECTS OF COMPARATIVE PULMONARY TOXICITY 2-30

3. QUALITATIVE EVALUATION OF THE DATA BASE 3-1

    3.1 GUIDELINES FOR SELECTIONS OF KEY STUDIES 3-1
        3.1.1 Human Data 3-1
            3.1.1.1 Epidemiologic Data 3-2

CONTENTS (continued)

Page

3.1.1.1.1 Assessment of exposure measures 3-2
3.1.1.1.2 Assessment of effect measures .. 3-3
3.1.1.1.3 Assessing the control of confounding and covariables 3-5
3.1.1.1.4 Summary 3-5
3.1.1.2 Nonepidemiologic Data 3-5
3.1.1.2.1 Clinical studies 3-6
3.1.1.2.2 Case reports 3-6
3.1.1.3 Intraspecies Variability and Identifying Sensitive Subgroups 3-6
3.1.2 Animal Data 3-10
3.1.2.1 Appropriateness of Species as a Model for Humans 3-11
3.1.2.2 Study Design 3-12
3.1.2.3 Study Validity and Relevance to Extrapolation 3-13
3.1.3 Summarizing the Evidence 3-14
3.2 TOXICOLOGICAL ISSUES IN DATA EVALUATION 3-16
3.2.1 Qualitative Evaluation of Dose Response and Dose Effect Data 3-16
3.2.1.1 Relationship to the Uncertainty Factor Approach 3-16
3.2.2 Selecting Effect Levels: Inhalation-Specific Issues 3-21
3.3 DEFICIENT DATA BASES AND ALTERNATIVE SOLUTIONS 3-24
3.3.1 Guidance on Evaluating a Data Base for Completeness 3-25
3.3.2 Historical Use and Limitations of Occupational Exposure Limit Values 3-25

4. QUANTITATIVE METHODOLOGICAL PROCEDURES 4-1

4.1 PROCEDURES ADDRESSING LIFETIME EXPOSURE 4-1
4.1.1 Approach for RfD Estimation 4-1
4.1.1.1 Minimum Criteria 4-9
4.1.1.2 Calculation of Human Equivalent Concentrations 4-10
4.1.1.2.1 Dose conversion: units 4-12
4.1.1.2.2 Dose adjustments for discontinuous exposure protocols 4-13
4.1.1.2.3 Dosimetry: particles 4-14
4.1.1.2.3.1 Respiratory tract effects 4-17
4.1.1.2.3.2 Extrarespiratory effects 4-19
4.1.1.2.3.3 Assumptions and default values 4-20

CONTENTS (continued)

Page

4.1.1.2.4 Dosimetry: Gases and vapors ... 4-22
4.1.1.2.4.1 Respiratory tract effects 4-23
4.1.1.2.4.2 Extrarespiratory effects 4-24
4.1.1.2.4.3 Assumptions and default values 4-28
4.1.1.3 Route-to-Route Extrapolation 4-29
4.1.1.4 Issues for Further Investigation 4-31
4.1.2 Approach for RfD Estimation Using Human Data 4-31
4.1.2.1 Introduction 4-31
4.1.2.2 Selecting the Threshold Estimate 4-32
4.1.2.3 Defining the Exposure Level 4-33
4.1.2.4 Uncertainty Factors for Human Data 4-33
4.2 PROCEDURES FOR ESTIMATING PARTIAL LIFETIME EXPOSURES....... 4-34
4.2.1 Acute 4-34
4.2.2 Approach for Subchronic Inhalation RfD Estimation (RfD_{si}) 4-34
4.2.3 Issues Requiring Further Investigation 4-37
4.3 CRITERIA FOR SPECIFYING LEVEL OF CONFIDENCE 4-37

5. REFERENCES 5-1

APPENDIX A: NOVEL APPROACHES TO THE ESTIMATION OF REFERENCE DOSE (RfD) A-1

APPENDIX B: USE OF PHARMACOKINETIC DATA IN RISK ASSESSMENT, SELECTED EXAMPLES B-1

APPENDIX C: ADVERSE RESPIRATORY HEALTH EFFECTS (HUMAN) C-1

APPENDIX D: CRITERIA FOR ASSESSING THE QUALITY OF INDIVIDUAL EPIDEMIOLOGICAL STUDIES D-1

APPENDIX E: CRITERIA FOR ASSESSING THE QUALITY OF INDIVIDUAL ANIMAL TOXICITY STUDIES E-1

APPENDIX F: CRITERIA FOR CAUSAL SIGNIFICANCE F-1

APPENDIX G: CHOICE OF TOXICITY DATA G-1

APPENDIX H: CALCULATION CURVES FOR RDDR AND AN EXAMPLE APPLICATION OF DOSIMETRIC ADJUSTMENT H-1

CONTENTS (continued)

Page

APPENDIX I: DERIVATION OF AN APPROACH TO DETERMINE HUMAN EQUIVALENT CONCENTRATIONS FOR EXTRARESPIRATORY EFFECTS OF GAS EXPOSURES BASED ON A PB-PK MODEL USING SELECTED PARAMETER VALUES I-1

TABLES

Number		Page
2-1	Comparative airway anatomy as revealed on casts	2-5
2-2	Normal surface airway epithelium: cell types	2-14
2-3	Some specific lung cell types and their function	2-16
2-4	Main species differences in epithelial cells and glands	2-18
3-1	Prevalence of subgroups hypersusceptible to effects of common pollutants	3-8
3-2	Proposed approach for summarizing the evidence from diverse data	3-16
3-3	Human data for use in health risk assessment	3-17
4-1	Four types of response levels (ranked in order of increasing severity of toxic effect) considered in deriving inhalation reference doses (RfD_is) for systemic toxicants	4-2
4-2	Response levels considered in deriving inhalation RfDs in relationship to empirical severity rating valves (ranks are from lowest to highest severity)	4-3
4-3	Guidelines for the use of uncertainty factors in deriving reference dose (RfD)	4-5
4-4	Minimum data base for both high and low confidence in the RfD	4-40
A-1	Various effects levels and their definitions used in Figure A-2	A-4
B-1	Absorption of 1,3-butadiene by inhalation following a 6-hour exposure period	B-1
B-2	Recovery of ^{14}C-tetrachloroethylene radioactivity after inhalation exposure for 6 hours to Sprague-Dawley rats	B-4
H-1	RDDR values by mass median diameter and standard deviation for rats	H-6
H-2	Summary of systemic toxicity NOAELs for ep(a)oxide observed in Fisher rats	H-16
I-1	Definition of symbols	I-4

FIGURES

Figure		Page
2-1	Schematic representation of selected parameters influencing regional deposition of particles in the respiratory tract	2-7
2-2	Regional deposition of monodisperse aerosols by indicated particle diameter for mouth breathing (alveolar and tracheobronchial) and nose breathing (alveolar)	2-8
2-3	Schematic representation of selected parameters influencing regional deposition of gases in the respiratory tract	2-10
2-4	An example of the log-normal distribution function of an aerosol	2-20
2-5	Plot of same aerosol as in Figure 2-4 on log-probability paper. The curves illustrate the various size parameters that can be computed using the Hatch-Choate equations	2-20
4-1	Flowchart for calculation of human equivalent concentrations	4-11
4-2	Relationship of partition coefficients to periodicity in F344 rat arterial blood for (90-days) subchronic and chronic exposure regimens of 6 hours/day, 5 days/week	4-27
A-1	Effect-dose-duration plot of all relevant human and animal oral toxicity data for methoxychlor	A-3
A-2	Hypothetical dose-response data for slight body weight decrease or liver necrosis in rats and dogs	A-7
H-1	Schematic of the integration of aerosol distribution and deposition efficiency curves for calculation of Regional Deposited Dose (RDD)	H-2
H-2	RDDR of the rat:human by particle diameter (MMAD) for the tracheobronchial region	H-12
H-3	RDDR of the rat:human by particle diameter (MMAD) for the pulmonary region	H-13
H-4	RRDR of the rat:human by particle diameter (MMAD) for the total respiratory system on (A) normal augmenters and (B) mouth breathers	H-14

FIGURES

Figure Page

H-5 RDDR of three species to the human by particle diameter (MMAD) for the TH region. Guinea pig 1 and 2 refer to calculations using different lung surface area data H-18

H-6 The relationship between $t_{1/2p}$ and $t_{1/25}$ upon the bioavailability of a single deposited dose of inhaled particulate matter over a 730-day period H-21

I-1 Schematic of the physiologically based pharmacokinetic model assumed to describe the uptake and distribution of inhaled compounds I-2

I-2 Plot of $NOAEL_{HEC}$ vs. $NOAEL_{A}$ for the rat for four possible methods (proposed, established, similar and optimal) of determining $NOAEL_{HEC}$ estimates as defined in the text. The inhaled compound is dichloromethane 1-13

I-3 Plot of $NOAEL_{HEC}$ vs. $NOAEL_{A}$ for the mouse for four possible methods (proposed, established, similar and optimal) of determining $NOAEL_{HEC}$ estimates as defined in the text. The inhaled compound is dichloromethane 1-13

LIST OF ABBREVIATIONS

ADI	Acceptable daily intake
bw	Body weight
CNS	Central nervous system
D_{ae}	Aerodynamic equivalent diameter
D_{ar}	Aerodynamic resistance diameter
DNA	Deoxyribonucleic acid
D_p	Particle diameter
DWEL	Drinking water equivalent level
FEL	Frank-effect level
FEV_1	Forced expiratory volume at one second
FVC	Forced vital capacity
GI	Gastrointestinal
HA	Health advisory
i.v.	Intravenous
kg	Kilogram
LEL	Lowest-effect level
LOAEL	Lowest-observed-adverse-effect level
LOEL	Lowest-observed-effect level
MF	Modifying factor
mg	Milligram
μg	Microgram
μm	Micrometer
MMAD	Mass median aerodynamic diameter
NOAEL	No-observed-adverse-effect level
NOEL	No-observed-effect level
PEL	Permissible exposure level
ppm	Parts per million
RDD	Regional deposited dose
RDDR	Regional deposited dose ratio
RfD_i	Chronic inhalation reference dose
RfD_{sic}	Subchronic inhalation reference dose

RNA	Ribonucleic acid
σ_g	Geometric standard deviation
TLV	Threshold limit value
UF	Uncertainty factor
URT	Upper respiratory tract
V_T	Tidal volume

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The authors wish to acknowledge the scientific guidance and support provided by Dr. Fred Miller (ORD, OHR, HERL), Ms. Margaret Menache (NSI-Technology Services Corporation), Dr. Daniel Guth (OAR, OAQPS, PAB) and Dr. Judith Bellin (Risk Assessment Forum).

The authors thank Bette Zwayer and Carol Haynes (ECAO-Cin) for diligently and graciously preparing the first drafts of the manuscript and Judith Olsen for excellent editorial support. The authors thank Ivra Bunn, Lynette Davis, Patricia Felix, Miriam Gattis, and Lorrie Godley of NSI-Technology Corporation (RTP) for preparing the workshop, revised and final drafts and the final document.

The authors would also like to express a special tribute to the late Director of ECAO-Cin, Dr. Jerry F. Stara. Without his vision and guidance, this effort would not have been possible.

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GLOSSARY

Activity Median Diameter (AMD)

Refers to the median of the distribution of radioactivity, toxicological, or biological activity with respect to particle size.

Acute Exposure

A one-time or short-term exposure with a duration of less than or equal to 24 hours.

Aerodynamic Diameter

Term used to describe particles with common inertial properties to avoid the complications associated with the effects of particle size, shape, and physical density.

Aerodynamic equivalent diameter (D_{ae})

"Aerodynamic diameter" generally used. The diameter of a unit density sphere ($\rho_p = 1$ g/cm^3) having the same settling velocity (due to gravity) as the particle of interest of whatever shape and density. Refer to Raabe (1976) for equation.

Aerodynamic (viscous) resistance diameter (D_{ar})

The "Lovelace" definition for aerodynamic diameter. Characteristic expression based on terms describing a particle in the Stokes' regime. Refer to Raabe (1976) for equation.

Aerosol

All-inclusive term. A suspension of liquid or solid particles in air.

Critical Effect

The first adverse effect, or its known precursor, that occurs as the dose rate increases.

Chronic Exposure

Multiple exposures occurring over an extended period of time, or a significant fraction of the animal's or the individual's lifetime.

Diffusion Diameter

Diameter of a sphere having the same diffusion mobility as the particle in question. $D_p < 0.5$ μm.

Forced expiratory volume (FEV_1) at one second

The volume of air which can be forcibly exhaled during the first second of expiration following a maximal inspiration.

Forced vital capacity (FVC)

The maximal volume of air which can be exhaled as forcibly and rapidly as possible after a maximal inspiration.

Generation

Refers to the branching pattern of the airways. Each division into a major daughter (larger in diameter) and minor daughter airway is termed a generation. Numbering begins with the trachea.

Henry's Law Constant

The law can be expressed in several equivalent forms, a convenient form being: $C_g = HC_l$ where C_g and C_l are the gas-(g) and liquid-(l) phase concentrations. The constant (H) is the ratio at equilibrium of the gas phase concentration to the liquid-phase concentration of the gas (i.e., moles per liter in air/moles per liter in solution).

Lowest-Effect Level (LEL)

Same as Lowest-Observed-Adverse-Effect Level.

Lowest-Observed-Adverse-Effect Level (LOAEL)

The lowest exposure level at which there are statistically or biologically significant increases in frequency or seventy of adverse effects between the exposed population and its appropriate control group.

Mass Median Aerodynamic Diameter (MMAD)

Mass median of the distribution of mass with respect to aerodynamic diameter. Graphs for these distributions are constructed by plotting frequency against aerodynamic diameters.

Modifying Factor (MF)

An uncertainty factor that is greater than zero and less than or equal to 10; its magnitude reflects professional judgment regarding scientific uncertainties of the data base or study design not explicitly treated by the uncertainty factors (e.g., the number of animals tested). The default value for the MF is 1.

No-Observed-Adverse-Effect Level (NOAEL)

An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control. Some effects may be produced at this level, but they are not considered as adverse, nor precursors to specific adverse effects. In an experiment with several NOAELs, the regulatory focus is primarily on the highest one, leading to the common usage of the term NOAEL as the highest exposure without adverse effect.

Portal-of-Entry Effect

A local effect produced at the tissue or organ of first contact between the biological system and the toxicant.

Reference Dose (RfD)

An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation reference dose is for continuous inhalation exposures and is appropriately expressed in units of mg/m^3. It may be expressed as mg/kg/day, in order to compare with oral RfD units, utilizing specified conversion assumptions.

Regional Deposited Dose (RDD)

The deposited dose (mg/cm^2 of lung region surface area per minute) calculated for the region of interest as related to the observed effect (i.e., calculated for the tracheobronchial region for an effect concerning the conducting airways).

Regional Deposited Dose Ratio (RDDR)

The ratio of the regional deposited dose in the animal species of interest (RDD_A) to that of humans (RDD_H). This ratio is used to adjust the exposure effect level for interspecies dosimetric differences.

Reserve Volume

Volume of air remaining in the lungs after a maximal expiration.

Respiratory Bronchiole

Noncartilagenous airway with lumen open along one side to alveoli; when walls are completely alveolarized it is usually referred to as an alveolar duct. Essentially absent in rats.

Stokes' Law

The total drag force or resistance of the medium due to fluid motion relative to the particle is the sum of form and friction drag. When particle motion is described by this equation, it is said to be in the Stokes regime.

Subchronic Exposure

Multiple or continous exposures occuring over about 10% of an experimental species lifetime, usually over 3 months.

Terminal Bronchiole

Noncartilagenous airway that conducts airstream to respiratory bronchiole.

Threshold

The dose or exposure below which a significant adverse effect is not expected. Carcinogenicity is thought to be a nonthreshold endpoint, thus, no exposure can be presumed to be without some risk of adverse effect. Noncarcinogenicity is presumed to be a threshold endpoint, thus, some exposures are presumed to be without risk of adverse effects.

Tidal Volume (V_T)

Volume of air inhaled/exhaled during normal breathing

Uncertainty Factor (UF)

One of several, generally 10-fold factors, used in operationally deriving the Reference Dose (RfD) from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population; (2) the uncertainty in extrapolating animal data to the case of humans; (3) the uncertainty in extrapolating from data obtained in a study that is of less-than-lifetime exposure; (4) the uncertainty in using LOAEL data rather than NOAEL data; and (5) the inability of any single study to adequately address all possible adverse outcomes in humans.

1. INTRODUCTION

1.1 DEVELOPING BENCHMARK VALUES IN THE U.S. ENVIRONMENTAL PROTECTION AGENCY

This document focuses on toxicological issues central to the development of an approach for the quantitative assessment of risks of health effects other than cancer and gene mutations for inhaled agents and to the development of an interim methodology for the estimation of inhalation reference doses (RfD_is). An inhalation reference doses is an estimate (with uncertainty spanning perhaps an order of magnitude) of continuous exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious effects during a lifetime. The documentation discusses criteria and information to be considered in selecting key studies for inhalation RfD derivation, provides an overview of the respiratory system and its intra- and interspecies variables, and discusses areas of uncertainty and data gaps in relation to the proposed interim methodology.

The U.S. Environmental Protection Agency (U.S. EPA) has a history of advocating the evaluation of scientific data and calculation of Acceptable Daily Intake (ADI) values for noncarcinogens as benchmark values for deriving regulatory levels to protect exposed populations from adverse effects. The Office of Pesticide Programs used the concept of ADI for tolerance estimates of pesticides in foodstuffs. The Office of Health and Environmental Assessment used ADI values for characterizing levels of pollutants in ambient waters (Federal Register, 1980). The National Research Council (1977, 1980) recommended the ADI approach to characterize levels of pollutants in drinking water with respect to human health; the U.S. EPA Office of Drinking Water has adopted the National Academy of Sciences (NAS) approach.

The U.S. Environmental Protection Agency (1987a) has developed guidelines for the evaluation of available data pertaining to xenobiotics for purposes of developing RfDs analogous in intent to the ADI approach for oral exposures. While similar to ADIs in intent, RfDs are based upon a more rigorously defined methodology. In addition, guidelines for developing risk assessments have been promulgated for mutagenicity, carcinogenicity, mixtures, teratogenicity and reproduction, and for estimation of exposure (Federal Register, 1986a through e). Draft guidelines also are available for female and male reproductive toxicity (Federal Register 1988a,b).

The U.S. EPA's effort to develop these RfDs involves several parallel efforts: (1) development of guidelines for establishing levels of confidence in RfDs; (2) verification of existing RfDs; and (3) identification and analysis of toxicologic data pertinent to the development of RfDs.

In order to adapt this approach to derive inhalation benchmark values analogous to those existing for the oral RfD, it is necessary to develop the scientific basis for estimating inhalation values, develop guidelines, and encourage broad scientific review.

The Agency recognizes that regional, state, and local health protection departments need uniform and scientifically sound risk assessment procedures for the estimation of benchmark inhalation values. The proliferation of diverse risk assessment values for inhalation exposure and the resulting confusion this has caused attests to the importance of a consensus approach to uniform guidelines. It is the intention of the Agency that the interim RfD approach described will be useful to many in their risk management programs as one piece of the risk assessment process. The approach outlined is not intended to discourage novel or more sophisticated risk assessment procedures when sufficient data are available. The recognized deficiencies in this RfD approach and other novel approaches under development are described in Appendix A, and examples of the use of pharmacokinetic data in risk assessment are provided in Appendix B. Current research and ongoing projects to refine inhalation dose estimates are outlined in Appendices H and I. The interim RfD methodology proposed is consistent with previous Agency approaches, however, and is considered suitable for implementation.

The issue paper on Occupational Exposure Limit (OEL) values, developed by the Inhalation Technical Panel of EPA's Risk Assessment Forum, discusses the history, use, and limitations of OELs as surrogates for ambient exposure RfD values (U.S. Environmental Protection Agency, 1989).

1.2 GENERAL PRINCIPLES OF NONCANCER TOXICITY RISK ASSESSMENT*

Toxic endpoints other than cancer and gene mutations are often referred to as "noncancer toxicity" because of effects on the function of various organ systems. Most chemicals that produce noncancer toxicity do not cause a similar degree of toxicity in all organs, but usually demonstrate major toxicity to one or two organs. These are referred to as the target organs of toxicity for that chemical (Doull et al., 1980). Generally, based on our understanding of homeostatic and adaptive mechanisms, noncancer toxicity is treated as if there is an identifiable threshold (both for the individual and for the population); however, the Agency is aware of the difficulties in the identification of population thresholds (Gaylor, 1985) below which effects are not observable. This threshold approach distinguishes noncancer endpoints from carcinogenic and mutagenic endpoints, which are often treated operationally as nonthreshold processes.

The individual threshold hypothesis holds that a range of exposures from zero to some finite value can be tolerated by the organism without adverse effects. For example, there could be a large number of cells performing the same or similar function whose population must be significantly depleted before an adverse effect is seen. Further, it is often prudent to focus on the most sensitive members of the population and, therefore, regulatory efforts are made to keep exposures below levels at which the more sensitive individuals in the population would be expected to respond.

Empirical observation generally reveals that as the dosage of a toxicant is increased, the toxic response (in terms of severity and/or incidence of effect) also increases. This dose-response relationship is well-founded in the theory and practice of toxicology and pharmacology. Such behavior is exemplified by three types of data: (1) quantal responses, in which the number of responding individuals in a population increases; (2) dose-graded responses, in which the severity of the toxic response within an individual increases with dose; and (3) continuous responses, in which changes in a biological parameter (e.g., body or organ weight) vary with dose.

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*This text is excerpted and adapted from U.S. Environmental Protection Agency (1987a).

The majority of previous risk assessment efforts for noncancer health effects have been directed at oral exposures. Human data appropriate for quantifying risk assessments for oral exposure are limited; therefore, the majority of these assessments have relied on animal data. These animal studies typically reflect situations in which exposure to the toxicant has been carefully controlled, and the problems of heterogeneity of the exposed population and concurrent exposures to other toxicants have been minimized. In evaluating animal data, a series of professional judgments are made involving, among other things, consideration of the scientific quality of the studies. Presented with data from several animal studies, the risk assessor first seeks to identify the animal model that is most relevant to humans, based on compatibility of biological effects using the most defensible biological rationale; for instance, by using comparative metabolic, pharmacokinetic, and pharmacodynamic data. In the absence of a clearly most relevant species, however, the most sensitive species is used as a matter of science policy at the U.S. EPA. For inhalation RfDs, the most sensitive species is the species that shows an adverse effect at an exposure level which when dosimetrically adjusted, results in the lowest human equivalent concentration. Guidance for full utilization of human data has not been extensively explored because of the limited availability of relevant human oral data. However, for the inhalation route, a substantially greater quantity of human data useful to risk assessment is anticipated. Subsequent sections of this document will explore the issues associated with human data that are particularly relevant to the inhalation route of exposure.

In the simplest terms, an experimental exposure level is selected from a given study of a species representing the highest level tested at which no adverse effect was demonstrated. The inhalation methodology requires conversion of these "No-Observed-Adverse-Effect Levels" (NOAELs) observed in animals to human equivalent concentrations ($\text{NOAEL}_{\text{HEC}}$s) before the data array and effect levels can be evaluated and compared. A chemical may elicit more than one toxic effect (endpoint) in tests of the same or different duration (acute, subchronic, and chronic exposure studies), even in one test species. In general, $\text{NOAEL}_{\text{HEC}}$s for these effects will differ. The critical toxic effect used in the dose-response assessment is the one generally characterized by the lowest $\text{NOAEL}_{\text{HEC}}$. The $\text{NOAEL}_{\text{HEC}}$ is the key datum gleaned from the study of the dose-response relationship and, traditionally, is the first basis for the scientific evaluation of the benchmark level in the RfD approach. This approach is based, in part, on the assumption that if the critical toxic effect is prevented, then all toxic effects are prevented.

The RfD is a benchmark dose operationally derived from the $\text{NOAEL}_{\text{HEC}}$ of the critical effect by consistent application of generally order of magnitude uncertainty factors (UFs) that represent the second basis for the scientific evaluation of the RfD. The uncertainty factors reflect potential extrapolation uncertainty between the characteristics of the study situation and the projection to daily exposure of humans. The RfDs and the composite uncertainty factors vary in magnitude depending upon the particular study; for example, a valid NOAEL for chronically exposed healthy humans is normally divided by a UF of 10-fold to extrapolate to a more susceptible population. In addition, a modifying factor (MF), which is based on a professional judgment of the entire data base of the chemical, may be included. That is:

$$\text{RfD (or ADI)} = \text{NOAEL}_{\text{HEC}}/(\text{UF} \times \text{MF})$$

Inhalation RfDs pertain to continuous exposures for a lifetime. If exposure assumptions are changed and appropriate toxicologic data utilized, benchmark values may be calculated for exposure durations of less than a lifetime (see Section 4.2). An evaluation of the adequacy of presently used uncertainty factors in extrapolating from subchronic to chronic inhalation exposure is an outstanding issue to be addressed by the Risk Assessment Forum.

The Agency is attempting to standardize its approach in determining RfDs. This standardization will include statements on the confidence that the evaluators have in the RfD. High confidence is an indication that the RfD is unlikely to change as more data become available because there is consistency among the toxic responses observed in different sexes, species, study designs or in dose-response relationships. It is recognized, however, that increasingly sophisticated tests may change the perspective of evaluation. Often, high confidence is associated with RfDs that are based on human data for the exposure route of concern. Low confidence indicates that the RfD may be especially vulnerable to change if additional chronic toxicity data become available.

1.3 STATE-OF-THE-ART APPLICATIONS TO THE DEVELOPMENT OF THE INHALATION RfD METHODOLOGY

All risk assessments involve some degree of reliance upon assumptions, which substitute for unavailable quantitative information and thereby impart varying degrees of uncertainty in the risk assessment methodology. However, as state-of-the-art research and health risk science progresses, the precision of risk assessments will be improved, insofar as these advancements are incorporated into the assessments. Risk assessments ultimately serve as the basis for personal or governmental risk management decisions on safeguarding health and have consequential economic impacts. This makes it imperative that scientific advancements in risk assessment be made and that they be appropriately incorporated into risk assessment processes, including the derivation of inhalation RfDs. Based on this, the current inhalation RfD methodology is termed "interim," in view of planned future updating as advancements in risk assessment are made.

The Office of Research and Development (ORD) is conducting a rigorous research program to improve the scientific basis of risk assessments. When key information becomes available from this program, as well as relevant research from other institutions, it will be incorporated into the inhalation RfD methodology. This must be balanced against the necessity of a certain degree of consistency in risk assessment procedures, to improve the feasibility of broad regulatory application of the assessments. Therefore, the Office of Health and Environmental Assessment, ORD, will regularly evaluate scientific advancements in the field and make recommendations for significant improvements in the inhalation RfD methodology. Every two years, these recommendations are expected to be presented to an expert panel of EPA and extramural scientists for peer review. Modifications in the methodology will be made as appropriate. If research advancements having a striking impact on the methodology were to occur prior to this two-year recurring review, then the timing of the process would be altered appropriately.

As generic issues arise during the verification sessions of the inhalation RfD workgroup, they will be sent to a Risk Assessment Forum made up of an appointed technical panel of experts for review and resolution. The technical panel of the Risk Assessment Forum then will provide recommendations and guidance on such issues. This mechanism has provided useful input to the oral RfD methodology to date and is anticipated to provide refinements to the inhalation RfD methodology as well.

This interim methodology will be buttressed by a technical support document providing tabulated Regional Deposited Dose Ratios (RDDRs) for various species which will be produced in the near future. These ratios are used to adjust animal experimental exposure concentrations to human equivalent concentrations as discussed in Chapter 4 and Appendix H. The technical support document will provide a detailed description of their derivation and limitations of their application. Research also is already underway to provide a second technical support document of Regional Retained Dose Ratios (RRDRs). These ratios will integrate clearance functions into the deposited values for estimates more appropriate to assessing chronic exposure conditions.

At the time of the two-year review, it is expected that research advancements on uptake modeling of gases (discussed in Chapter 4 and Appendix I) will provide guidance on dosimetric adjustments for different categories of gases. Continued work on hygroscopic particle modeling may provide chemical-specific adjustment factors or a revised default condition for this category of aerosols.

Other ORD research projects anticipated to have significant impact on the methodology include: (1) guidance on the limitations and application of physiologically-based pharmacokinetic model parameters to route-to-route extrapolation, and (2) approaches for less-than-lifetime assessment. An appropriate characterization of activity patterns of human ventilatory levels also is expected to be developed so that the aerosol deposition and gas uptake models can be utilized to provide more realistic estimates of probable human exposure.

In summary, one objective of the Interim Inhalation RfD methodology is that it always be scientifically based, and thus, the methodology should be considered dynamic. Pertinent issues and their solutions will be incorporated as identified on a continuing basis. Periodic peer review will provide quality assurance. These actions will make the methodology sufficiently reliable to serve as one of the key bases for decisions on protecting the public health.

2. CONCEPTUAL BASIS FOR INHALATION RISK ASSESSMENT METHODOLOGY

As discussed in the introduction, there are some fundamental differences to be considered in performing risk assessments of inhalation exposures to chemicals and of oral exposures. The primary differences are the degree to which the complex relationship between exposure dose and dose delivered to the target site can be addressed and the more common occurrence of portal-of-entry effects. Both of these are described below to serve as a basis for criteria that must be added to the oral RfD methodology to facilitate development of inhalation RfDs.

2.1 FACTORS CONTROLLING COMPARATIVE INHALED DOSE

It is anticipated that the derivation of inhalation RfDs will not be as straightforward as that of oral RfDs, given the dynamics of the respiratory system and its diversity across species. The various species used in inhalation toxicology studies do not receive identical doses in comparable respiratory tract regions when exposed to the same particle or gas concentration (Brain and Mensah, 1983). The biologic endpoint or health effect may be more directly related to the quantitative pattern of mass deposited within the respiratory tract than to the exposure concentration. Regional deposition pattern determines not only the initial lung tissue dose but also the specific pathways and rates by which the inhaled agents are cleared and redistributed (Schlesinger, 1985).

This section presents the issues associated with the major factors controlling the deposition pattern, which are: (1) respiratory anatomy and physiology (Section 2.1.1); and (2) the physicochemical characteristics of the inhaled agent (Section 2.1.2). Section 2.1.3 presents restrictions imposed by experimental procedures and technology, and working assumptions that affect the two major controlling factors.

The factors that control inhaled dose are discussed relative to the significant mechanisms by which particles and gases may initially be deposited or taken up in the lung. For particles this includes inertial impaction, sedimentation (gravitational), diffusion, interception, and electrostatic precipitation, while mechanisms important for gases include convection, diffusion, chemical reaction, and solubility. Detailed consideration of these mechanisms is beyond the scope of this discussion. The reader is referred elsewhere for more extensive discussions of particle deposition (U.S. Environmental Protection Agency, 1982; U.S. Environmental Protection Agency, 1986b; Hatch and Gross, 1964; Raabe, 1979; Hinds, 1982; Lippmann and Schlesinger, 1984) and gas absorption (U.S. Environmental Protection Agency, 1986c; Fiserova-Bergerova, 1983; Overton, 1984; Overton and Miller, 1988).

It must be emphasized that dissection of the factors that control inhaled dose into discrete discussions is deceptive and masks the dynamic nature of the intact respiratory system. For example, although deposition in a particular respiratory region will be discussed separately from the clearance mechanisms for that region, retention (the actual amount of inhaled agent found in the lungs at any time) is determined by the relative rates of deposition and clearance. Retention and the toxicologic properties of the inhaled agent are presumably related to the magnitude of the pharmacologic, physiologic, or pathologic response. Thus, although the deposition, clearance mechanisms, and physiochemical properties of the agent are described in distinct sections, assessment of the overall toxicity requires integration of the various factors into a dynamic picture.

Future improvements in this process will be accomplished in the area of extrapolation modeling (Miller et al., 1983a; Fiserova-Bergerova, 1983). This involves determining the effective dose delivered to the target organ of various species and the sensitivity of the target organ to that dose. Once such dosimetry has been established, and species sensitivity accounted for, the effective pollutant concentration in animals can be quantitatively related to concentration responses in humans. Extrapolation models should incorporate parameters such as species anatomical and ventilatory differences, metabolic processes, and the physicochemical properties of the pollutant and should be physiologically based upon the factors that govern transport and removal of the pollutant.

In the interim, a qualitative knowledge and application of how regional deposition and disposition patterns, and metabolism of an inhaled dose may differ between humans and experimental animals commonly used in inhalation toxicology investigations will provide more accurate cross-species dosimetric extrapolations.

2.1.1 Respiratory Anatomy and Physiology

The respiratory systems of humans and various experimental animals differ in anatomy and physiology in many quantitative and qualitative ways. These variations affect air flow patterns in the respiratory system, and in turn, the deposition of an inhaled agent, as well as the retention of that agent in the system. The variations in anatomy and physiology will be discussed according to respiratory regions and branching patterns, clearance mechanisms, and cell types. Clearance mechanisms as used here include processes such as the mucociliary escalator, solubilization in various compartments, uptake, and metabolism.

2.1.1.1 Respiratory Regions and Branching Patterns. The respiratory system in both humans and experimental animals can be divided into three regions on the basis of structure, size, and function: nasopharyngeal, tracheobronchial, and pulmonary (alveolar). The retained dose of an inhaled agent in each of these regions is governed by the individual species anatomy (e.g., airway size and branching pattern) and physiology (e.g., breathing rate and clearance mechanisms).

Airway size and branching pattern affect the aerodynamics of the respiratory system in the following ways:

- The airway diameter affects the aerodynamics of the flow and the distance from the agent molecule or particle to the airway surface.
- The cross-sectional area of the airway determines the airflow velocity for a given volumetric flow.
- Diameter and branching pattern variations affect the mixing between tidal and reserve air.

Differences in airway sizes and branching between species thus result in significantly different patterns of gas transport and particle deposition.

2.1.1.1.1 Effect on aerosol deposition mechanisms. Air flow in the extrathoric region is characterized by high velocity and abrupt directional changes. Thus, the predominant deposition mechanism in the extrathoracic region is inertial impaction. Changes in airstream direction or magnitude of air velocity streamlines or eddy components do not affect airborne particles due to their inertia. Large particles (>5 µm) are more efficiently removed from the airstream in this region.

Impaction remains a significant deposition mechanism for particles larger than 2.5 µm aerodynamic equivalent diameter (D_{ae}) in the larger airways of the tracheobronchial region and competes with sedimentation, with each mechanism being influenced by mean flow rate and residence time, respectively. As the airways successively bifurcate, the total cross-sectional area increases. This increases airway volume in the region and the air velocity is decreased. With decreases in velocity and more gradual changes in air flow direction as the branching continues, there is more time for gravitational forces (sedimentation) to deposit the particle. For particles ≈4 µm D_{ae}, a transition zone between the two mechanisms, from impaction to predominantly sedimentation, has been observed (U.S. Environmental Protection Agency, 1982). This transition shifts toward smaller particles for nose breathing.

Differences in airway size and branching pattern are a major source of interspecies variability in inhaled dose for the tracheobronchial region. Larger airway diameter results in greater turbulence for the same relative flow velocity (e.g., between a particle and air). Therefore, flow may be turbulent in the large airways of humans, while for an identical flow velocity, it would be laminar in the smaller experimental animal. Relative to humans, experimental animals also tend to have tracheas that are much longer in relation to their diameter. This could result in increased deposition in humans because of the increased likelihood of laryngeal jet flow extending into the bronchi. Humans are characterized by a more symmetrical dichotomous branching than that found in most laboratory mammals, which have highly asymmetrical branching (monopodial). The more symmetrical dichotomous pattern in humans is susceptible to deposition at the carina because of its exposure to high air flow velocities toward the center of the air flow profile. These comparative airway anatomy differences are summarized in Table 2-1.

Sedimentation becomes insignificant relative to diffusion as the particles become smaller. Deposition by diffusion results from the random (Brownian)

TABLE 2-1. COMPARATIVE AIRWAY ANATOMY AS REVEALED ON CASTS

	Gross Structure					Typical Structure (Generation 6)			
Mammal/ Body Mass	Left Lung Lobes	Right Lung Lobes	Airway Branching	Trachea L/D[a] (cm)	Major Airway Bifurcations	Airway L/D (ratio)	Branch Angles (Major Daughter/ Minor Daughter) (degrees)	Typical Number of Branches to Terminal Bronchiole	Respiratory Bronchioles
Human/70 kg	upper and lower	upper, middle and lower	relatively symmetric	12/2	Sharp for about the first 10 generations, relatively blunt thereafter	2.2	11/33	14-17	About 3-5 orders
Rhesus monkey/2 kg	superior, middle, and inferior	superior, middle,and inferior, azygous	monopodial	3/0.3	Mixed blunt and sharp	2.6	20/62	10-18	About 4 orders
Beagle dog/ 10 kg	apical, intermediate, and basal	apical, intermediate, and basal	strongly monopodial	17/1.6	Blunt tracheal bifurcation, others sharp	1.3	8/62	15-22	About 3-5 orders
Ferret/ 0.61 kg	NR[b]	NR	strongly monopodial	10/0.5	Sharp	2.0	16/57	12-20	About 3-4 orders
Guinea pig/ 1 kg	superior and inferior	superior, middle and inferior	monopodial	5.7/0.4	Very sharp and high	1.7	7/76	12-20	About 1 order
Rabbit/ 4.5 kg	superior and inferior	cranial, middle, caudal and postcaval	strongly monopodial	6/0.5	Sharp	1.9	15/75	12-20	About 1-2 orders
Rat/0.3 kg	one lobe	cranial, middle, caudal, and postcaval	strongly monopodial	2.3/0.26	Very sharp and very high throughout lung	1.5	13/60	12-20	Rudimentary
Golden hamster/ 0.14 kg	superior and inferior	cranial, middle caudal, and postcaval	strongly monopodial	2.4/0.26	Very sharp	1.2	15/63	10-18	About 1 order

[a] L/D = Length/diameter ratio

[b] NR = Not reported

Source: Phalen and Oldham, 1983; Patra, 1986; Crapo, 1987 motion of very small particles caused by the collision of gas molecules in air. The terminal settling velocity of a particle approaches 0.001 cm/s for a unit density sphere with a physical diameter of 0.5 μm, so that gravitational forces become negligible. The main deposition mechanism is diffusion for a particle whose physical (geometric) size is <0.5 μm. Impaction and sedimentation are the main deposition mechanisms for a particle whose size is greater than 0.5 μm. Hence, D_{ae} = 0.5 μm is convenient for use as the boundary. Although this convention may lead to confusion in the case of very dense particles, most environmental aerosols have densities below 3 g/cm^3 (U.S. Environmental Protection Agency, 1982). Diffusional deposition is important in the small airways and in the pulmonary region where distances between the particles and airway epithelium are small.

These mechanisms for particle deposition in the respiratory tract are schematically represented in Figure 2-1. Experimental deposition data and extrapolated estimates on humans that illustrate these same concepts are shown by the curves for pulmonary (alveolar) and tracheobronchial deposition in Figure 2-2. Deposition fraction is shown plotted against particle diameter. It is important to note that over half of the total mass of a typical ambient mass distribution would be deposited in the extrathoracic region during normal nasal breathing, with most of this being coarse particles (U.S. Environmental Protection Agency, 1986B). With mouth-only breathing, the regional deposition pattern changes dramatically, with extrathoracic deposition being reduced and both tracheobronchial and pulmonary deposition enhanced. Oronasal breathing (partly via the mouth and partly nasally), however, typically occurs in healthy adults while undergoing moderate to heavy exercise. Thus, the appropriate activity pattern of subjects for risk assessment estimation remains an important issue. Miller et al. (1988) recently examined extrathoracic and thoracic deposition as a function of particle size for ventilation rates ranging from normal respiration to heavy exercise. A family of deposition estimate curves were generated as a function of breathing pattern. Anatomic and functional differences between adults and children are likely to yield complex interactions with the major mechanisms affecting respiratory tract deposition, again with implications for risk assessment. Age-dependent dosimetric adjustments may be possible, pending data availability for children.

2.1.1.1.2 Effect on gas deposition and uptake. The major processes affecting gas transport involve convection, diffusion, absorption, solubility, and chemical reactions. These mechanisms are schematically represented in

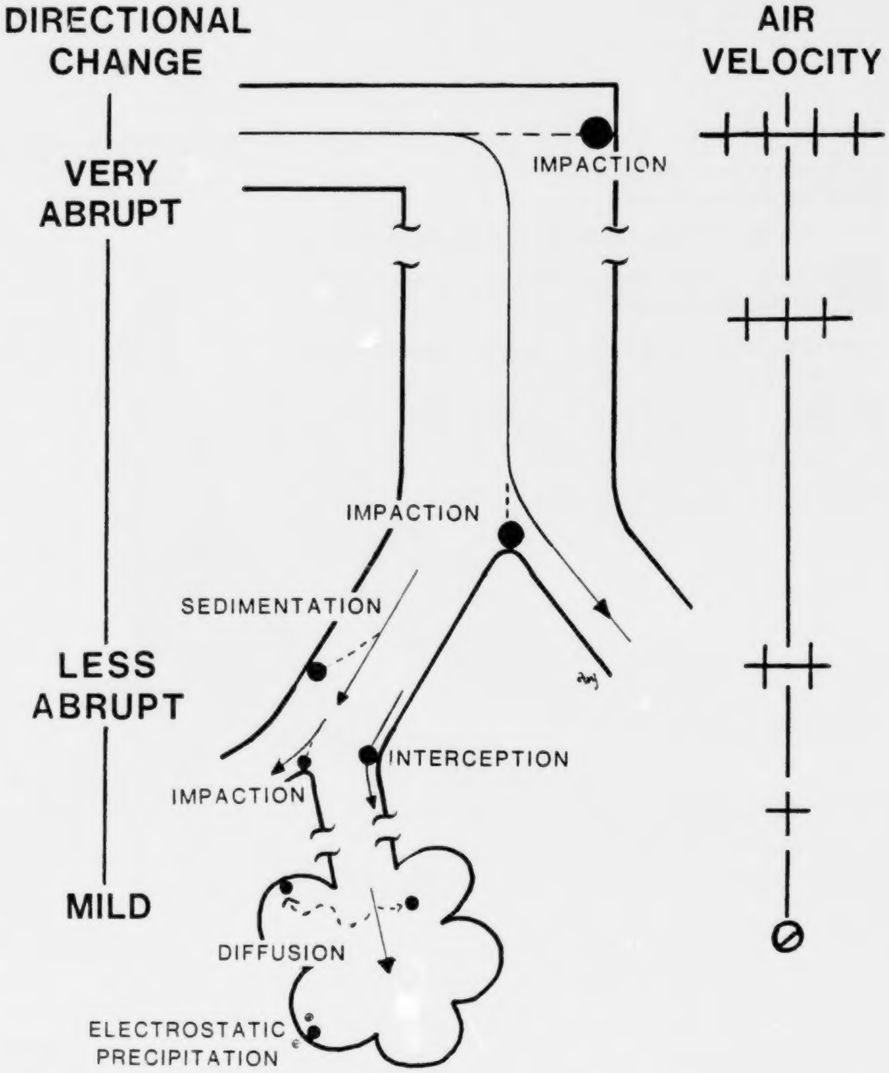
DIRECTIONAL CHANGE
VERY ABRUPT
LESS ABRUPT
MILD
AIR VELOCITY
IMPACTION
IMPACTION
SEDIMENTATION
INTERCEPTION
IMPACTION
DIFFUSION
ELECTROSTATIC PRECIPITATION
0

Figure 2-1. Schematic representation of selected parameters influencing regional deposition of particles in the respiratory tract.

Source: Adapted from Casarett, 1975; Raabe, 1979; Lippmann and Schlesinger, 1984.

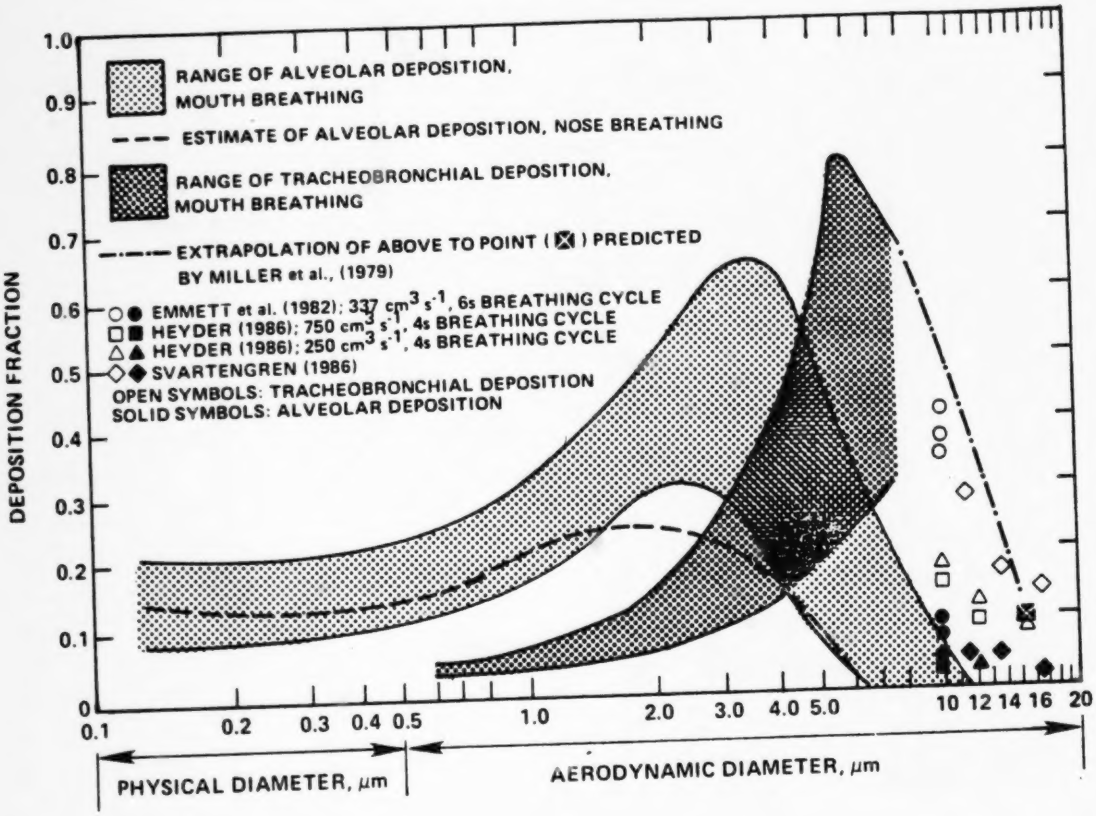

Figure 2-2. Regional deposition of monodisperse particles by indicated particle diameter for mouth breathing (alveolar and tracheobronchial) and nose breathing (alveolar). Deposition is expressed as fraction of particles entering the mouth or nose. The alveolar band indicates the range of results found by different investigators using different subjects and flow parameters for pulmonary (alveolar) deposition following mouth breathing. The tracheobronchial (TB) band indicates intersubject variability in deposition over the size range measured by Chan and Lippmann (1980). The extrapolation of the upper bound of the TB curve in the larger particle size range also is shown and appears to be substantiated by data listed in the legend.

Source: U.S. Environmental Protection Agency, 1986b.

Figure 2-3. The bulk movement of inspired gas in the respiratory tract is induced by a pressure gradient and is termed convection (U.S. Environmental Protection Agency, 1982). Convection can be broken down into components of advection (horizontal movement of a mass of air relative to the airway wall) and eddy dispersion (air mixing by turbulence so that individual fluid elements transport the gas and generate flux). Molecular diffusion is superimposed at all times on convection (bulk flow) due to local concentration gradients. Absorption removes gases from the lumen and affects concentration gradients.

The average concentration of a gas in a tube (i.e., an "idealized" airway) can be described by one-dimensional convection and dispersion. A pulse of substance moves down a tube with an average air velocity equal to the medium's (air's) average velocity, and its spread in the axial direction is governed by an effective dispersion coefficient that can be described by Fick's law of diffusion (Overton, 1984). This effective dispersion coefficient is larger than the molecular diffusion coefficient except in the pulmonary region. As illustrated in Figure 2-3, perpendicular transport in this region can carry a gas molecule into the alveoli, but because of the alveolar walls, there is no net axial transport as is present in the central channel. The average axial transport is slowed because only a fraction of the molecules in the cross-sectional average can move axially, resulting in a dispersion process with a dispersion coefficient less than the molecule coefficient. The coefficient is a function of the molecular diffusion coefficient, the total air volume, and the generation's alveolar airspace volume (Overton, 1984).

Molecules are transferred from the flowing gas into the liquid layer lining the airway wall by molecular diffusion. A simple description for this process postulates a thin, stagnant layer based on the assumption that the air velocity becomes very small as the air-liquid interface is approached. Transfer through this layer depends on the gas-phase diffusion coefficient, layer thickness, and the gas concentrations at the boundaries of the layer. If the molecules are absorbed, then the concentration of the gas in the diffusion layer is decreased at the liquid boundary. As the ability of the liquid to remove the gas increases, the relative concentration at the gas-liquid boundary decreases, and the mass transfer from the gas phase to the liquid phase increases. For poorly soluble, hydrophobic, and nonreactive gases, little gas is removed by the airways. The transport and chemistry into the adjacent liquid and tissue layers will be described in Section 2.1.2.2, which describes

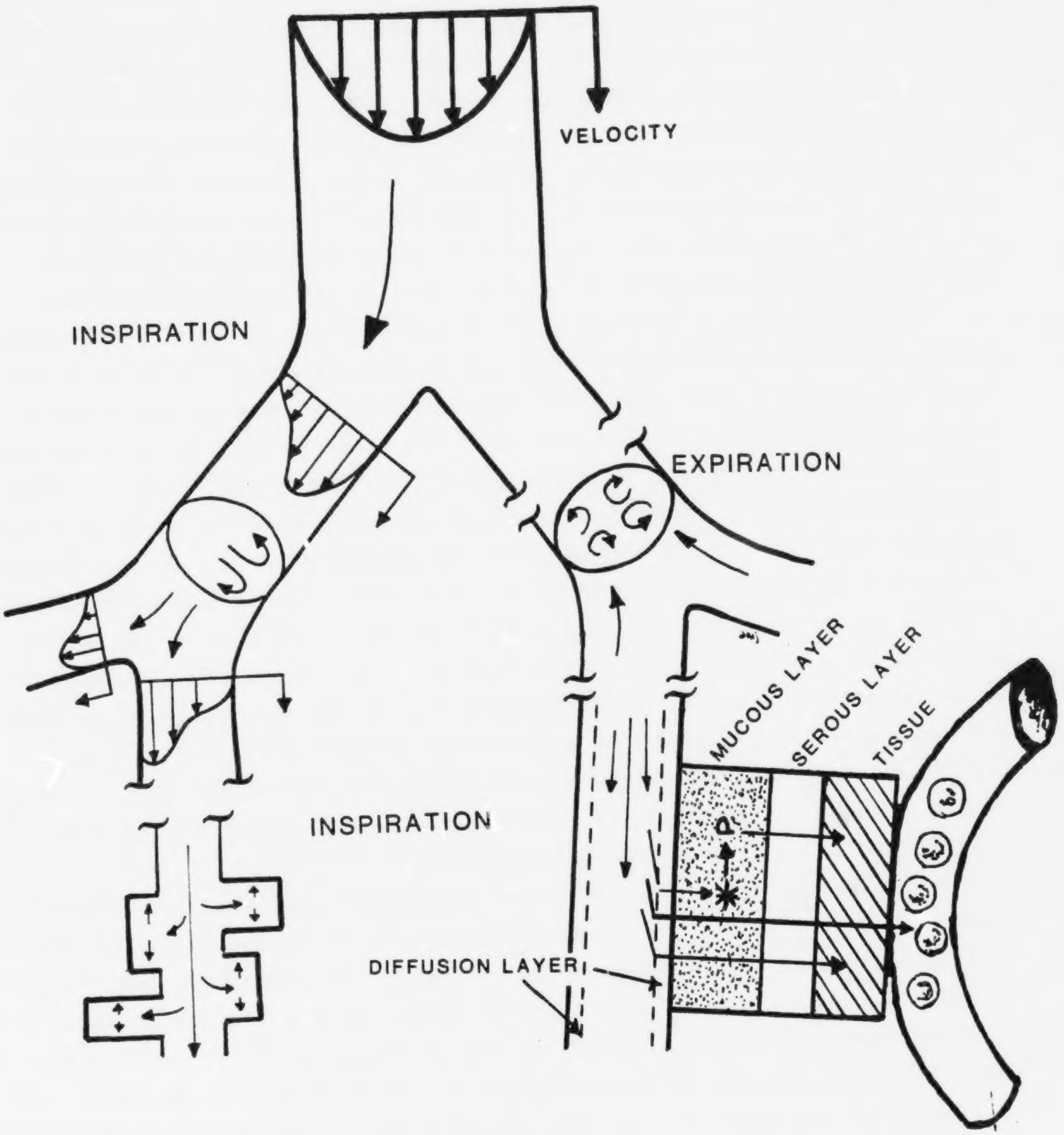
VELOCITY
INSPIRATION
EXPIRATION
MUCOUS LAYER
SEROUS LAYER
TISSUE
INSPIRATION
DIFFUSION LAYER

Figure 2-3. Schematic representation of selected parameters influencing regional deposition of gases in the respiratory tract.

Source: Overton, 1984.

the physicochemical characteristics of gases and vapors. These next layers can serve as a "sink" to help "drive" the delivery of gas across this layer. Capillary blood flow (i.e., perfusion) is important to the gas uptake in that it removes the gas or its chemical reaction products on the other side of these liquid and tissue layers. Thus, addressing species differences in alveolar ventilation and cardiac output is critical to estimate initial absorbed dose. The importance of regional differences (e.g., the distance from the air to the capillaries in the tracheobronchial region is 7-20 times that in the pulmonary region [Overton and Miller, 1988]) and interspecies differences in the anatomic relationship of the airspace to capillary blood should be considered. Transfer also is enhanced by a reduction in diffusion layer thickness that is dependent on the nearby rate of airflow; the higher the flow velocity, the thinner the layer, again emphasizing the significance of airway morphology.

To attempt to model the effects that the intricate morphological structure of the respiratory tract has on the nature of gas mixing and flows, representations of the mechanical mixing imparted by tube bifurcations, turbulence, and secondary flows due to molecular diffusion must be formulated. Location identity, diameter, and length are considered to be the relevant measurements for gas transport (Overton, 1984). Because of the morphology of the respiratory tract and air flow patterns, the relative contribution of these gas transport processes is a function of location and point in the breathing cycle (i.e., depth and rate) (U.S. Environmental Protection Agency, 1982; Overton, 1984). The interspecies differences in the nature and structure of the respiratory tract, as summarized in Table 2-1, critically influence the differences in transport and deposition of gases across species. The airways also show a considerable degree of intraspecific size variability and are most likely the primary factor responsible for the deposition variability seen within single species (Schlesinger, 1985). Additionally, gender influences airway anatomy, and age has dramatic influences on respiratory dynamics.

The differences in airway anatomy summarized in this Section (2.1.1) form the structural basis for the species differences in gas and aerosol deposition. Extensive investigations that resulted in the quantitation of the effects that these differences have on the deposition of insoluble particles have resulted in the dosimetry adjustments for inhaled dose that are outlined in Section 4.1.1.3. Current research on interspecies differences for gas distribution and deposition should result in similar adjustments for gaseous inhaled agents. In addition to the structure of the lung, the regional thickness and composition of the airway epithelium (a function of cell types and distributions) is an important factor in gas absorption, and contributes to the solubility and extent of reaction of the gas. Other anatomic and physiologic factors that influence gas uptake include: (1) ventilation, which affects the tidal volume and ventilation to perfusion ratios; (2) body build, which affects the volume of distribution (including cardiac output and tissue volume); and (3) metabolic capacities. These are all factors to evaluate when estimating inhaled dose, interpreting injury response, and extrapolating effects between species.

2.1.1.2 Clearance Mechanisms and Cell Types. Inhaled material is removed from the respiratory tract by clearance mechanisms, which vary depending on the site of deposition and the properties of the inhaled agent. For gases, the sequence in which anatomic sites are affected appears to be more dependent on concentration than on exposure duration. However, at a given local anatomic site and at a specific concentration, the stages in the pathogenesis of the lesion relate to the duration of exposure (U.S. Environmental Protection Agency, 1986c). The speed and efficiency by which the agents are cleared can be critical determinants of their toxic potential. Rapid removal lessens the time available to cause critical damage to the pulmonary tissue and to permit systemic absorption of agents that have target organs other than the lung (Menzel and Amdur, 1986). The mechanisms involved include (1) exhalation of volatiles; (2) mucociliary transport; (3) macrophage phagocytosis; (4) chemical reactions; (5) metabolism by various cell types; and (6) dissolution and absorption into the blood, lymphatic, or lung fluids.

The transport and chemical uptake mechanisms for gases described in Section 2.1.2.2 are a function of respiratory tract region. Conceptually, a gas can move from the airway lumen, through the liquid lining layer, through the tissue layer, through the capillary endothelium, to reach the blood. This passage is influenced by the physiochemical properties of the gas as well as the biochemistry and thickness of the layers between the lumen and blood. For example, a very highly reactive gas may not reach the blood if it reacts biochemically with mucus and the mucus has sufficient volume (thickness) to serve as a sink. This same gas may not react with the saturated lipid of surfactant, and if deposited significantly in the pulmonary region, could reach alveolar tissue. The thickness and efficiency of the epithelial barrier also influences absorption. Both of these main factors (liquid lining and epithelial barrier) are present in all species but have species-specific differences, only a few of which have been quantified. Mucous is a complex secretion with contributions from various epithelial cells. The numbers and distribution of these cells may affect the composition and properties of the mucous, which in turn interacts with the physicochemical properties of the agent. The species differences in the thickness of the alveolar epithelial cells could account for variations observed in the diffusion of gases into the bloodstream (Crapo et al., 1983). The lung also is a very efficient excretory organ for volatile organic chemicals after the exposure ceases or is lowered. The efficacy of pulmonary excretion correlates indirectly with the saturated vapor pressure of the chemical.

Clearance of particles involves different mechanisms. Particles deposited on the anterior nares are cleared by mechanical processes such as nose wiping, blowing (humans), or sneezing (animals/humans). Particles in this area can have long biological half-lives. Those deposited in the nasopharynx or oropharynx, however, are swallowed within minutes and passed through the esophagus down to the gastrointestinal tract.

Particles deposited in the tracheobronchial region are transported out of the respiratory tract by the mucociliary system, an interaction between the mucous secretions and the cilia that provide the mechanisms of movement. Such transport occurs along the area from the larynx to the terminal bronchioles. Insoluble particles are transported up to the esophagus where they are swallowed. The rate of this transport also affects the gas transport mechanisms in the diffusion layer. The rate varies with the depth of the airways (greater velocities in the proximal airways) and across species. Generally, the biological half-lives of particles deposited in the tracheobronchial region are on the order of hours.

Clearance from the pulmonary region of the lung takes the longest, usually a rapid phase of hours, and slower phases with biological half-lives of days, months, or years, depending on particle size and solubility. Processes contributing to the removal of deposited materials in this area include phagocytosis by macrophages and removal by the blood or lymph, and dissolution into the blood, lymph, or lung fluids (Johanson and Gould, 1977).

The numerous cell types found in different species also contribute to the varying clearance patterns from the respiratory regions and differences in the nature of the response. Table 2-2 presents the distributions of various cell

TABLE 2-2. NORMAL SURFACE AIRWAY EPITHELIUM: CELL TYPES

	Humans	Monkey	Dog	Ferret	Guinea Pig	Rabbit	Rat	Hamster	Mouse
Epithelial									
Ciliated	+	+	+	+	+	+	+	+	+
Mucous	+	+	+	+	+	+	+	+	+
Serous	a	-	-	-	-	-	b	c	-
Clara	+	+	+	+	+	+	+	+	+
Endocrine	+	+	-	-	+	+	+	+	+
Type I	+	+	+	+	+	+	+	+	+
Type II	+	+	+	+	+	+	+	+	+
Transitional	d	-	-	-	-	-	e	g	f
Special type	h	-	+	-	-	-	-	-	-
Brush	-	-	-	+	+	+	+	-	+
Intermediate	+	-	+	+	-	-	+	+	+
Basal	+	+	+	+	+	+	+	+	+
Migratory									
Lymphocyte	+	i	-	-	-	+	+	+	+
Globule leukocyte	-	i	i	-	-	-	+	-	-
Mast cell	h	+	i	-	+	-	-	-	-
Macrophage	+	(+)	+	(+)	(+)	(+)	+	(+)	(+)
Neural									
Neuroepithelial body	+	+	-	-	-	+	+	-	+
Nerve terminals	h	+	-	+	+	+	+	+	j

+ = reported present;

(+) = not specifically reported in sources cited;

- = unidentified; a = fetal tissue;

b = in specific pathogen-free rats;

c = only young animals;

d = ciliomucous, mucoserous, endocrine-mucous;

e = seromucous;

f = ciliomucous, seromucous;

g = ciliomucous;

h = not in "normal" biopsy material;

i = "migratory cell";

j = bronchiolus only

Source: Jeffery, 1983; Crapo et al., 1983 types across species commonly used in inhalation toxicologic investigations. Recent investigation have also shown species differences in cellular organization at the terminal respiratory bronchioles/alveolar duct junctions and in the ultrastructure of the same cell type across species (St. George et al., 1988). The possible functions of these cell types are provided in Table 2-3, while the differences seen in the cell types across species are summarized in Table 2-4. Such species differences are important to consider when determining if the animal is an appropriate model for the chemical's mechanism of action. For example, the rat may be an inappropriate species for the evaluation of hypersensitivity because of its lack of mast cells.

Due to the major influence of respiratory tract structure on the dosimetry of inhaled agents, extrapolation from animal models to humans requires analysis of toxicological studies complicated by the complexity and diversity of the respiratory tract across species. Because of this, it is imperative that both similarities and differences across species in respiratory tract structure be incorporated into modeling efforts. More recent data on cellular morphometrics and interspecies differences in cell populations (Mercer and Crapo, 1987; St. George et al., 1988) will aid in dosimetry adjustments for clearance, metabolism, and uptake. As an example, modeling for the metabolic capacity of the human lung instead of considering it only as a physical barrier can result in disparate estimates of extrapulmonary dose. Epithelial secretions in response to injury may recruit scavenger cells such as polymorphonuclear leukocytes, which can biotransform inhaled agents. Different species have different amounts, distribution, and levels of cytochrome P-450 of their Clara cells, which could account for differences in metabolism of some agents.

Interspecies differences in clearance rates have the potential to alter the estimated dose to a given species and thus could significantly alter the derived RfD_i. Differences in clearance rates now are being calculated into the interspecies ratios used for dosimetric adjustment of the exposure concentrations used in RfD_i derivation for estimation of a retained dose (see Chapter 4 and Appendices H and I). Similar adjustments for differences in gas uptake due to differences in ventilation, perfusion, metabolism, and excretion are also warranted.

TABLE 2-3. SOME SPECIFIC LUNG CELL TYPES AND THEIR FUNCTION

Cell Types	Location and Function
Epithelium	
Clara cells	high metabolic activity; secretory; nonciliated; function not well-defined; may serve as precursor of goblet and ciliated cells
Ciliated cells	most common epithelial cells in airways; may secrete mucous-like substances; controls perciliary fluid
Type II alveolar cells	covers 3 percent of alveolar surface; secrete surfactant; replace injured Type 1 cells; high metabolic activity
Type I alveolar	large and covers considerable surface area per cell; covers $\geq$95 percent of alveolar surface; forms the alveolar epithelium and facilitates gas exchange; low metabolic activity; incapable of self-reproduction
Mucous	mucous-secreting
Serous	mucous-secreting; perciliary fluid; stem cell
Brush cells	chemoreceptor cells; preciliated
Globule leukocyte	immunoglobulin transportation; releases inflammatory mediators
Endocrine	secreto-and vaso-regulatory
Submucosal	
Goblet (mucous) cells	epithelial linings; common in trachea and bronchioles; contribute to mucous production
Serous cells	mucous-secreting; perciliary fluid; stem cell/ proliferative
Endocrine cells	secretes amines and neuropeptides
Lymphocytes	immunoresponsive
Myoepithelial	expulsion of mucous
Bronchoalveolar mast cells	migratory cells located throughout respiratory tract; release mediators of bronchoconstriction when antigens bind to IgE antibodies on surface

(continued on the following page)

TABLE 2-3. (continued)

Cell Types	Location and Function
Macrophage	phagocytic; secrete mediators of inflammatory reactions; modulate lymphocytes and otherwise participate in immune response
Endothelial cells	40 percent of lung parenchyma cells; metabolize blood-borne substances; proliferative
Fibroblasts (interstitial)	predominant in alveolar wall and constitutes the basement membrane; become activated during disease states and produce elastin and collagen; proliferation leads to fibrosis, modulation of growth, bronchial tone, and mucosal secretion

Source: Jeffery, 1983; Bowden, 1983; Marin, 1986; Nadel et al., 1985; Plopper et al., 1983; Burri, 1985; Brain, 1986.

2.1.1.3 Summary. This comparative overview of the complexity and diversity of the respiratory system in different species of mammals that are used in risk assessment, although difficult to use in a quantitative manner at this point, strongly suggests the potential for wide variation in deposited dose, cellular function, metabolism, and response to injury. Until the comparative morphometric and physiologic studies quantitate the functional implications of these differences, the risk assessor who is extrapolating across different species must choose results judiciously, based on a qualitative knowledge of comparative airway structure and function.

2.1.2 Physicochemical Characteristics of the Inhaled Agent

The physicochemical characteristics of the inhaled agent will influence the deposition and retention within the respiratory tract, translocation within the respiratory system, distribution to other tissues, and ultimately, the toxic effect. It is therefore important to consider characteristics of the inhaled agent as well when attempting to evaluate and extrapolate the effects of a particular exposure.

2.1.2.1 Particles. For a given particle exposure, the two most important parameters determining deposition are the mean diameter and the distribution of the particle diameters. The size and shape of the particles influence their aerodynamic behavior and, thus, their deposition. The definition of diameter for a spherical particle is unambiguous, but for irregular particles, a variety

TABLE 2-4. MAIN SPECIES DIFFERENCES IN EPITHELIAL CELLS AND GLANDS

Epithelial Morphology
- Thickness and pseudostratification
- Thickness and structure of "basement membrane"

- Mucous-secreting cells
  - number
  - histochemistry
  - predominant ultrastructure type

- Clara cells
  - morphology (smooth endoplasmic reticulum)
  - distribution

- Endocrine cell frequency

- Cilia
  - extent of coverage
  - structure of rootlet
  - lamellar bodies
  - glycogen stores

- Presence of brush cell

- Basal cells
  - number
  - shape
  - tonofilaments

Presence of Globule Leukocyte
- Innervation
  - extent
  - distribution
  - type

Gland Morphology
- Amount
- Distribution
- Main histochemical cell type
- Presence of collecting duct
- Innervation

*Source: Jeffery, 1983.

of definitions exist. Nonspherical particle size often is described by its aerodynamic properties. Fibrous material may be described by actual length, actual diameter, coil length, coil diameter, aspect ratio, or coil to aspect ratio.

Information about particle size distribution aids in the evaluation of the effective inhaled dose (Hofmann, 1982). Recommendations defining the particle size ranges for inspirability to the various regions have been published by an ad hoc working group of the International Standards Organization (1981). Particle size distribution should be provided to the risk assessor in addition to the particle diameter to more completely characterize the aerosol. For studies where total mass of inhaled particles is used in assessing health effects, it is appropriate to evaluate the particle size distribution in terms of mass, such as the mass median diameter. Figures 2-4 and 2-5 illustrate the distribution of various parameters used to characterize aerosol size.

It is useful to consider the particle's physical parameters that are responsible for the health effect of concern. The activity diameter of a particle may be the most appropriate expression of size for this purpose. This expression takes into account the "activity" of the physical property of the particle. For example, if the toxin is distributed only on the surface, then the activity median diameter is equal to the surface median diameter; calculations based on total mass would be inappropriate in such situations. If the toxicant is soluble, the surface area of the particle will influence the rate of dissolution since solubilization occurs at the surface. Such a situation needs to be understood better, especially for complex particles.

2.1.2.2 Gases and Vapors. The deposition site and rate of uptake of a volatile chemical are determined by its reactivity and solubility characteristics. Thus, the pharmacokinetics of gases and vapors are governed by:

- Rate of transfer from the environment to the tissue,
- Capacity of the body to retain the material, and
- Elimination of the parent compound and metabolites by chemical reaction, metabolism, exhalation or excretion.

As mentioned in Section 2.1.1.1.2, the transport processes in the liquid and tissue layers adjacent to the airway lumen influence the relationship of

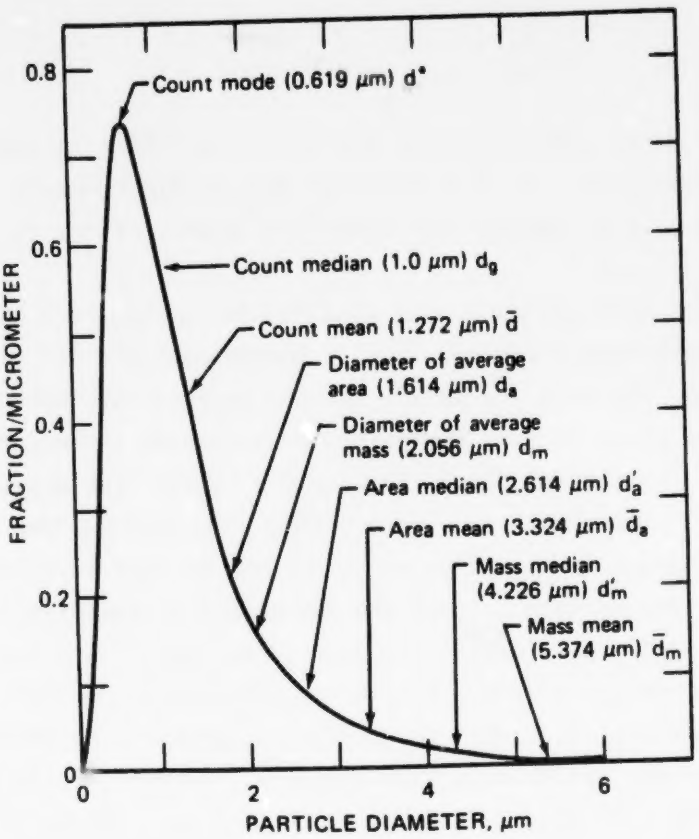

Figure 2-4. An example of the log-normal distribution function of an aerosol.

Source: Orr and Keng, 1976.

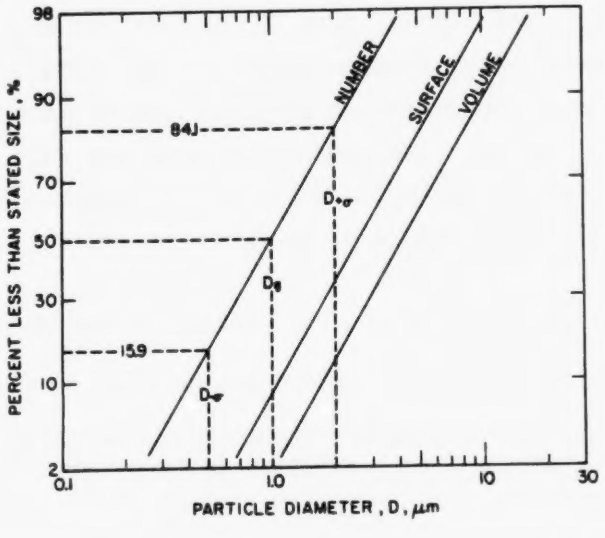

Figure 2-5. Plot of same aerosol as in Figure 2-4 on log-probability paper. The curves illustrate the various size parameters that can be computed using the Hatch-Choate equations.

Source: Marple and Rubow, 1980.

the gas with the air-liquid boundary. Physicochemical characteristics of the gas that contribute to the relative importance of these processes include its chemical reactivity and solubility.

The chemical reactions of the gas with both the liquid and tissue layers may be important. For example, reactions with the liquid layer could result in an increased flux from the airway but reduce (relative to no reactions) the delivery of the gas to the tissue. If the gas is the only toxic molecule, then this reaction would protect the tissue. Conversely, if the reaction products are toxic, then reactions with the tissue layer would increase the delivery of toxic molecules to the tissue (Overton, 1984). Chemical reactivity with the biological constituents of the tissue is similarly important to the gas' toxic potential to the lung tissue and to the amount of gas and reaction products that enter the blood for potential extrapulmonary toxicity. Theoretically, knowledge of all the chemical species involved and the reaction rates of the reactants and products is necessary to characterize a system for dosimetry. Sometimes the complexities may be reduced into relative classifications (e.g., slow, fast, instantaneous) using approximation techniques for time and spatial dependence (Overton and Miller, 1988). Gases that are not soluble or reactive are relatively inert to the airways and penetrate to the alveoli. Examples are nitrogen and volatile hydrophobic chemicals. The major factor driving the uptake of these gases is the removal of the gas from alveolar air by capillary blood. The concentration in alveolar air and capillary blood is generally considered to reach equilibrium. Thus, uptake of alveolar gases depends on air to blood partitioning, ventilation/perfusion, and air and blood concentrations.

For gases that are soluble, uptake is linearly related to solubility (Overton and Miller, 1988). There are many different expressions for the solubility of gases, differing in terms of units as well as in terms of what chemical form of the gaseous species in the liquid phase is related to the gas-phase quantities. As long as the concentration of dissolved gas is small, and the pressure and temperature is not close to the critical temperature and pressure, then Henry's Law is obeyed (Overton and Miller, 1988). It should be noted that the Henry's Law constant is independent of chemical reactions so that it relates the molecular form of the gas in water and air, and not the total quantity absorbed in water to air quantities. Considering the importance of chemical reactions as described above, solubilities as indicated by Henry's Law constants may not be appropriate to fully describe uptake. Further, extrapolation of Henry's Law constants from water data to biological fluids and tissues is not always appropriate, particularly for organic compounds.

Because uptake and disposition of inhaled vapors and gases are driven by the equilibration of their partial pressures in tissues with their partial pressures in ambient air, solubility may be aptly described by Ostwald solubility coefficients at body temperature. Ostwald solubility coefficients and partition coefficients (concentration ratios of the volatile chemical in two phases with equilibrated partial pressures) have the same values (Fiserova-Bergovera et al., 1984). The tissue-gas partition coefficient of a chemical has been shown to correlate with its fat-gas and blood-gas partition coefficients so that linear correlation equations may provide a useful means of estimating tissue-gas and blood-gas partition coefficients (Fiserova-Bergovera and Diaz, 1986).

Thus, a thorough consideration of both reactivity and solubility is needed when evaluating a gas for its absorption potential. Absorption generalizations based on molecular weight are not recommended. As an example, the difference in solubility between methanol and ethane, which have similar molecular weights, is a result of the presence of the hydroxyl group on methanol. Interspecies comparisons necessitate consideration of the effects of the differences in anatomy and physiology described previously, but it can generally be stated that the more soluble and less reactive the gas, the more similar the deposition will be between humans and animals. Interspecies differences in body fat induce interspecies differences in uptake and distribution of lipophilic chemicals.

The physicochemical gas characteristics of reactivity and solubility will interact with physiologic parameters such as pulmonary ventilation, cardiac output (perfusion), metabolic pathways, tissue volumes, and excretory capacities. The relative contribution or interaction of these is, in turn, affected by the exposure conditions (concentration and duration), so that as emphasized previously, integration of these various factors is necessary to estimate the deposited (on airway surfaces) and absorbed doses in order to assess toxicity.

2.1.3 Impact of Experimental Protocol

The techniques and measurements used in inhalation toxicology investigations may affect the exposure conditions or the interpretation of toxic effects, thereby altering the results used for risk assessment. Areas that introduce uncertainty into interspecies extrapolations of inhaled dose include measurement techniques, the definitions and underlying assumptions used in the procedures, and the exposure technology. Careful consideration should be given to each when estimating the effective inhaled dose.

2.1.3.1 Pharmacologic Effects of Agents. The test agents may affect lung ventilation. Administration of a chemical with narcotic properties will lower physical activity, while an irritant might increase movement. The test agent could also alter clearance mechanisms. All these states would affect deposition, uptake and relention of the dose. In addition, the agent could disrupt the immune system and render the animal more susceptible to disease during long-term testing, thereby altering the study results.

There are several examples of irritating or potentially anesthetic chemicals that can depress ventilation. Chang et al. (1983) reported a 40 percent decrease in minute volume in mice exposed to 15 ppm formaldehyde. This inhibition was maintained during the entire course of the daily exposure period. Ventilation was decreased to as little as 1/15 of resting values during exposure of mice to 10 ppm ozone, and to as little as 1/3 of resting values during exposure of mice to acrylate esters (Bruce et al., 1979).

2.1.3.2 Measurement Techniques. Since measurements of ventilation and breathing mechanics often are used to evaluate respiratory functional alterations or to estimate inhaled/retained dose, performance parameters of such measurements are critical to their interpretation. The patterns of respiration (breathing route, depth, and rate) affect the air flow characteristics which, in turn, influence the relationship between competing particle deposition mechanisms and the relative contribution of gas transport processes. The penetration depth of the exposure air is determined by the tidal volume (V_T), the airway caliber, and the ratio of functional residual capacity to total lung capacity (FRC/TLC). As the FRC/TLC increases, deposition would be expected to increase (Schlesinger, 1985). For example, rapid breathing often is associated with increased deposition of larger particles in the upper respiratory tract, as compared to slow, deep breathing. Thus, performance parameters include both the factors that influence the test species (including human) respiration characteristics and the performance limitations of the techniques.

2.1.3.2.1 Anesthesia. Anesthesia greatly influences the respiration characteristics of the test animal. This is a consideration when evaluating pulmonary function parameters for adverse effects. Prolonged anesthesia can compromise the respiratory system, altering normal function and response. Anesthesia also can alter the metabolism of the study compound. Anesthesia has been reported to interfere with autonomic control, produce atelectasis, decrease lung compliance, block reflex responses, and introduce an undesirable risk to animals committed to long-term toxicology studies (Dorato et al., 1983). These alterations in ventilation and breathing mechanics produced by anesthesia could have severe effects on the results of respiratory function measurements. This possibility provided the impetus to the development of procedures for measuring respiration in unsedated laboratory animals (Amdur and Mead, 1958; Mauderly et al., 1979). Data now are available on respiratory characteristics in sedated and unsedated animals; consideration of anesthesia should be included in data analysis to ensure appropriate comparisons.

2.1.3.2.2 Breathing pattern. Consideration should be given to the possible alteration of breathing pattern due to the exposure concentration which would, in turn, alter the delivered dose. Exposure of certain agents such as irritants may lead to concentration-dependent changes in pulmonary mechanics measurements (Costa and Tepper, 1988; Alarie, 1981). Correct quantification of inhaled dose may therefore require measurement of respiratory rate and tidal volume during the course of the exposure. Such differences in delivered "dose" correlated with the species-dependent differences have been reported for formaldehyde toxicity (Chang et al., 1983).

Although clinical exposures and respiratory measurements (at least the noninvasive ones for functional mechanics) will be done on nonsedated humans, the breathing pattern remains an important consideration. Experimental protocol often dictates the breathing pattern (i.e., nonspontaneous breathing) where a subject patterns his or her breathing to a metronome or is instructed to take a deep breath on every fifth inhalation. Since the efficiency of time-dependent deposition mechanisms is greater during inspiration than expiration, an ideal "academic" breathing pattern would keep the inspiration time/expiration time ratio (t_i/t_e) constant (Heyder et al., 1975). Relevance of this academic pattern to risk assessment, however, remains equivocal and most investigations do not attempt to maintain a constant ratio. Documentation of breathing patterns should be included in the experimental protocol and considered in the extrapolation of dose.

2.1.3.2.3 Equipment specifications. The equipment used will impart restrictions on any interpretation (i.e., limitations of sensitivity for exposure analysis) of investigative results. Any underlying assumption or limitation of the equipment used should be considered when evaluating test results. The reader is referred to Costa and Tepper (1988) for a discussion of pulmonary function testing principles, methods, and equipment limitations.

2.1.3.3 Definitions/Underlying Assumptions. Additional variability and uncertainty in evaluating available inhalation studies occur because investigators have used different definitions of various respiratory regions and have employed different methods to estimate total or regional deposition. For example, total deposition often is estimated by calculating the difference between the amount of compound in the inhaled air and that in the exhaled air. By making assumptions about mixing and dead space, estimates of regional deposition may be obtained using measurements of the compound concentration in different volume fractions of the expired air. As another example, the definition of upper respiratory tract in various studies has included any or all of the following anatomic regions: nasopharynx, oropharynx, larynx or upper trachea. In other studies, deposition values based on chemical or radiologic assays of tissues after exposure assume no particle translocation before or during dissection. Some investigators include measurement of material in the gastrointestinal tract (GI) in their reported value for upper respiratory tract deposition, while others ignore this translocation. The underlying assumptions and working definitions for different experimental conditions can contribute a large degree of variability in reported results. Conversion to some common basis will be necessary in order to calculate and accurately compare inhaled doses.

2.1.3.4 Exposure Technology. Generation of the compound under study and subsequent exposure also will affect the derived inhaled dose. Exact determination of the dose achieved in inhalation studies is a complex process. Proper generation, appropriate characterization, and accurate delivery of the test atmosphere are integral to this determination. Varieties and limitations of the available technology must be considered when evaluating the selection of methods and interpreting experimental results.

2.1.3.4.1 Inhalation modes. The various exposure techniques can be divided according to the extent to which the test species are exposed. The techniques range from whole body exposure at the one extreme to exposures limited only to the lower respiratory tract (Lippmann, 1980). These techniques include whole body, head-only, nose-only, nasal, oral, and tracheal cannula exposures, and tracheal instillations. Practical considerations such as economic feasibility, special precautions for safe and efficient performance, amount of material, test compound stability, exposure duration, and the measurements desired dictate the selection of an exposure technique for a given study design. For example, whole body exposure of laboratory animals in cages is the most common method to conduct chronic inhalation exposures for more than 1-2 hours per day, while nose-only exposures are most often used for short durations. A systematic investigation of the effects of these different delivery techniques on the regional deposition in various species is needed.

Wolff et al. (1982) studied the deposition and retention of 0.1 µm $^{67}Ga_2O_3$ aggregate aerosols in Fischer 344 rats following whole body and nose-only exposures. In this investigation, lung deposition for whole body exposures was similar to that for nose-only exposures (~15 percent of the inhaled particles). Due to preening, passage of material into the GI tract, however, was 1.6-fold greater for whole body exposures than with nose-only exposures. This could be important in cases where there is either a specific GI response (i.e., stomach lesions) or substantial GI absorption which may result in a systemic effect. Rotation of animals in whole body chambers is recommended and should be included in the experimental design (Griffis et al., 1981) to minimize dosimetric differences that would result if the aerosol was not uniformly distributed in the chamber. The effects of factors such as thermal and/or other stress upon animals in confinement tubes used for nose- or head-only exposures need to be considered, particularly since these factors may be species-dependent. For example, rats in confinement tubes for short exposures have been shown to have respiratory values and body temperatures that remain constant, while Syrian golden hamsters exhibit increasing ventilation and temperature (Raabe et al., 1973). Adaptation to exposure or measurements may be a function of behavior, such as ability to be trained (Mauderly and Kritchevsky, 1979), but in general, animals in confinement tubes or animals forced to breathe through mouthpieces will experience abnormal stress (Raabe et al., 1973). This should be accounted for in the experimental protocol. The tubes can be modified into plethysmographs to monitor respiratory function changes, or cooled to a constant temperature. The inhalation mode affects human exposures as well. Since the nasal passages are more efficient at removing particles (particularly for large particles) than the oral cavity, increased lung deposition of larger particles could occur through mouth breathing. This would affect both the amount and the size distribution of an inhaled aerosol. Even the specific configuration of the mouthpieces used in oral exposures can affect the extent of deposition (Schlesinger, 1985). Miller et al. (1988) showed that regional respiratory tract deposition of insoluble particles in humans is a complex function of breathing route, ventilatory level, and the particulate physicochemical and aerodynamic properties.

2.1.3.4.2 Generation and characterization. Just as the working definitions and underlying assumptions alter the interpretation of measurement techniques, the operative exposure level (e.g., for use in risk assessment, prediction models, etc.) of a test agent is a function of how its particulate composition (mean particle diameter and distribution) and gas concentration are expressed. Other specific characteristics (e.g., adequate test substance mixing in chamber, hygroscopicity, charge density) should be accounted for as part of this description. The soundness and interpretation of the animal data are dependent on the methods employed to generate and analyze the test atmosphere data since the methods influence deposition calculations.

The two most common ways in which particle size is expressed are the count median diameter (CMD) and mass median diameter (MMD). The toxicity of a material is most consistently related to its mass distribution. Measurement of mass has the further advantage of a minor quantitative error at the small end of the size spectrum. To assess risk, however, the activity diameter may be a more appropriate expression of particle size as discussed in Section 2.1.2.1. Methods of particle measurement include settling, filtration, wet and dry impingement, multiple impaction, electrical precipitation, thermal precipitation, centrifugation, and observation of optical effects. Each of these has its own principle of operation and limits of sensitivity which, in turn, affect the expression or characterization of the test aerosol. Fiber exposures are further complicated by the need to describe the aspect criteria and distributions. As discussed in the section on anatomy and physiology, certain mechanisms contribute to the deposition fraction in each respiratory region. Failure to account for characteristics such as hygroscopicity or charge density when generating an aerosol could change its deposition in certain regions. This variability in the aerosol characterization would be expressed as uncertainty in the risk assessment.

Gaseous contaminant atmospheres are usually somewhat easier to characterize. Delivered concentrations must be consistent across exposure location and duration and may be less than the generated concentration. If the gas is extremely reactive, loss due to reactions with the walls of the transport system (e.g., tubing) will occur. Losses due to decomposition or alteration of the test substance during some generation procedures also may be a factor. Gas flow rate (delivery) must be known, steady, and calibrated for the given gas since it is density-dependent. Analysis of the air is limited by the detection device specifications. If on-line analysis is not feasible, consideration should be given to the frequency of samples taken. The period between samples for intermittent analysis should be less than one-tenth of the total exposure time for any given day (McKenna, 1982).

For all generation and characterization of pollutants, periodic calibration of all measurement systems is a critical quality control/quality assurance step. This also needs to be considered when evaluating the study.

2.1.3.4.3 Exposure regimen. Extrapolation from one exposure regimen to another has uncertainties, most of which are not quantified. For most chemicals, either particles or gases, the quantitative relationship between concentration and duration of exposure is not studied. Some studies have indicated that the relationship is dependent on many factors, including (1) number of exposure hours per day; (2) the exposure scenario, that is, continuous vs. interrupted (e.g., 1 week of exposure, 1 week of air, 1 week of exposure, etc.), vs. intermittent (X hours per day, Y days per week) regimens; (3) time of endpoint assessment (i.e., acute vs. subchronic vs. chronic studies); (4) endpoint(s); and (5) the mechanisms of toxicity. Examples for particles and gases follow which illustrate some of the complexities involved in extrapolating across exposure scenarios.

The actual amount of particles or gas found in the lungs at any time is determined by the relative rates of deposition and clearance. The efficiencies of the deposition mechanisms are different in each region of the lung. The defense mechanisms and clearance rates for each of these regions also are different. Thus, it is expected that the kinetics of the toxic effect of an exposure will be influenced by the duration of exposure. There is experimental evidence for such a differential dependence of effect on exposure duration. Albert et al. (1971) showed that low single doses or early effects of repeated exposure to cigarette smoke were associated with acceleration of clearance rates in the tracheobronchial trees of both donkeys and humans. Heavier doses and long-term repeated exposures were associated with sporadic clearance, stasis intervals, and some retrograde movement. Unfortunately, there has not been a systematic comparison and quantification of differential clearance rates across species. This will be necessary before the effects of duration can be assessed in the same models or default values can be developed.

Ozone can be used as an illustration for gases since it has a large health effects data base. Kenoyer et al. (1981) showed that rats exposed to ozone for 4 hours showed delays in the early clearance and an acceleration in the late clearance rate of tracer particles. These investigators postulated that the delays in early clearance could be caused by effects that decrease mucous transport (e.g., decreased ciliary beat rate or change in mucous properties), while acceleration of the late clearance rate was most likely due to an increase in numbers or activities of alveolar macrophages. Rats exposed intermittently (7-8 hours/day to O_3 for approximately one week) had similar changes in lung antioxidant enzymes to animals exposed continuously (24 hours/day), even though the dose, as expressed as the product of concentration (C) and time (T) of exposure, was different (Mustafa and Lee, 1976). Monkeys exposed to O_3 for 18 months continuously, or for 9 months bimonthly for 18 months had some similar alterations in lung morphology; additional alterations were observed in the intermittent exposure group having a lower (C x T) (Tyler et al., 1985). Huang et al. (1988) has shown, using morphometric measurements of the proximal alveolar region of lungs of rats receiving prolonged low level exposures to of O_3, that the increase in the relative volume of Type I epithelial cells was related to the (C x T), whereas other morphometric indices were more dependent on concentration than on time.

For NO_2, the data base is equally complex on the exposure scenario issue. Using the mouse infectivity model (an index of antibacterial lung defenses), concentration was found to be more important than duration of exposure in causing the effect (Gardner et al., 1979). When a typical urban pattern of NO_2 was used (i.e., a baseline of continuous exposure to a low level of NO_2 on which were superimposed two 1-hour peaks of NO_2 each weekday), the study indicated that on a (C x T) basis, this regimen was not more toxic than a continuous exposure to the baseline level after a short period of exposure (Graham et al., 1987). After a chronic exposure, the spikes to the baseline increased the effects relative to the baseline exposure only (Miller et al., 1987a).

The topic of extrapolating across different exposure scenarios is beyond the scope of this document. However, the few examples provided illustrate the complexity of the issue. Risk assessors will have to consider the effects of exposure on a case-by-case basis and utilize default assumptions until the needed research data are available.

2.1.4 Summary

This Section (2.1) has provided an overview of critical anatomic and physiologic interspecies differences, significant physicochemical characteristics of an agent that should be considered when evaluating an exposure, and the experimental procedures which may influence exposure conditions and interpretations of toxic responses. It was intended to emphasize areas that should be given careful consideration and integration into an overall risk estimate when analyzing the data base used for the derivation of an inhalation reference dose. The next Section (2.2) discusses the significance of the lung as the portal-of-entry for inhalation exposure.

2.2 PORTAL-OF-ENTRY CONSIDERATIONS: ASPECTS OF COMPARATIVE PULMONARY TOXICITY

Inhalation represents a route of exposure in which a variety of interrelated factors influence not only the nature of the effects (portal versus systemic) but also the manner by which they occur. The influence of target cell populations in the respiratory tract on the nature of the response is a factor unique to the inhalation route of exposure. Unlike the liver, a first-pass organ in oral exposures that has a more homogenous population of limited types of cells, the respiratory tract has more than 40 cell types (Sorokin, 1970). Xenobiotics which exert their action by direct effects of the parent compound or by metabolites can manifest profound differences in the nature and degree of response, depending on the route of exposure.

The likelihood of adverse effects in the respiratory tract can be affected by (1) production, distribution, and reactivity of metabolites by and among specific cell types; (2) the degree to which detoxification systems are overwhelmed (e.g., glutathione depletion); (3) efficiency and sensitivity of repair processes (e.g., type II cell proliferation); (4) efficiency of clearance processes; (5) airway mechanics; and (6) mechanism of action (e.g., pharmacologic or immunologic) (Boyd, 1980; Calabrese, 1983; Gram et al., 1986; Thrush et al., 1982; Nadel et al., 1985; Marin, 1986).

There are numerous pulmonary defense systems that protect the respiratory tract. While some pulmonary defense systems are truly protective, it must be kept in mind that many "activate" inhaled agents and may be responsible for adverse effects. Pulmonary defense systems can be physical in nature (e.g., filtration of particles by nasal hair), mechanical (e.g., expiration), enzymatic, or cellular (e.g. phagocytosis).

Nasal hair can be envisioned as a first line of defense. However, trapping of agents in the nose can serve as a source of irritation and/or more serious adverse effects. Some agents (e.g., formaldehyde, acrolein) have been shown to cause severe lesions in nasal epithelial cells (Morgan et al., 1986). The mouth also can be envisioned as another first-line defense system. Mouth-breathing in humans can result in solubilization of vapors in saliva and deposition of particles. Swallowing can reduce pulmonary exposure but increase presentation of the agent systemically via gastrointestinal tract absorption.

Once an agent penetrates to the tracheobronchial region, agent deposition and/or solubilization occurs in the mucous blanket covering the surface epithelium. Clearance is discussed in Section 2.1.1.2.

Metabolism of potentially toxic inhaled compounds is achieved by a variety of enzyme reactions involving oxidation, reduction, hydrolysis, and conjugation. The enzymes may work individually, concurrently, or consecutively to detoxify or, in some cases, toxify inhaled foreign compounds (Ohmiya and Mehendale, 1984; Minchin and Boyd, 1983; Dahl et al., 1987). These enzymes may vary in activity across species and organs (Ohmiya and Mehendale, 1984; Ziegler, 1980; Tynes and Hodgson, 1985; Plopper et al., 1983; Litterst et al., 1975).

The oxidation, reduction, and hydrolysis reactions are catalyzed primarily by the cytochrome P-450 and FAD containing monooxygenases. The cytochrome P-450 isoenzymes are ubiquitous hemoproteins located in the endoplasmic reticulum of a variety of cells and are responsible for the oxidation of foreign compounds. Recent studies have elucidated isoenzyme specificity, inducibility, catalytic activity, and localization in the rabbit and rat lung (Domin and Philpot, 1986; Vanderslice et al., 1987). Until recently, it was thought that the cytochrome P-450 isoenzymes were the only primary monooxygenases in the lung. However, recent studies have shown that the FAD-containing monooxygenases play an important role in detoxification of foreign compounds.

The Clara cells lining the respiratory and terminal bronchioles are thought to be the primary site of cytochrome P-450 because of the presence of endoplasmic reticulum. However, the ultrastructure of the Clara cell varies across species (Plopper et al., 1980). In the ox, cat, and dog, more than 60% of the cytoplasmic volume is glycogen with a relatively small proportion of the cell volume containing endoplasmic reticulum or mitochondria. Thus, species differences in Clara cell ultrastructure can be reflected in significant differences in xenobiotic metabolism potential (Plopper et al., 1983; St. George et al., 1988). Differences in localization of cytochrome P-450 activity have been suggested as a likely basis for some differences in respiratory tract toxicity (O'Brien et al., 1985).

Individually or in concert with the cytochrome P-450 isoenzymes, conjugation reactions are catalyzed by the GSH-S-transferases which transform potentially toxic parent compounds or activated metabolites into nontoxic water soluble compounds. The cofactor required for these reactions is the tripeptide, glutathione (GSH). GSH is synthesized in the lung, as well as in other major organs, and also is reduced from the oxidized state (GSSG) to the reduced state (GSH) by GSH reductase. Under extreme conditions of GSH depletion in the lung, it has been hypothesized that extrapulmonary GSH is mobilized and transported to the lung from the liver (Berggren et al., 1984). GSH has been identified in isolated Type II epithelial cells, Clara cells, and ciliated cells of the lung, but it is not known if it is present in all pulmonary cells. GSH also is the cofactor utilized by the enzyme GSH peroxidase. GSH peroxidase catalyzes the metabolism of hydrogen peroxide and organic peroxides formed by the ozonization of unsaturated fatty acids. Other key antioxidant components in the lung include ascorbic acid, alpha-tocopherol, superoxide dismutase, and catalase (Massaro et al., 1988).

A variety of other cellular defense mechanisms can be marshaled which can diminish or enhance toxic insult. Certain cell types can be stimulated to release mediators, such as mast cell release of histamine. Histamine can cause bronchoconstriction, which can be protective, by limiting the amount of pollutant inhaled, or can be toxic, in terms of limiting oxygen uptake. Synthesis or metabolism of prostaglandins also can affect airway and vascular caliber. The chemotactic factors released can recruit phagocytic cells involved in clearance. It should be recognized that the respiratory tract contains a variety of different cell types that possess different metabolizing potential and are distributed in a manner which varies among species. A list of common cell types and their function is provided in Table 2-3 in Section 2.1.2.1. Macrophages, for example, constitute a cellular protection system and not only protect inner surfaces of the respiratory tract from damage caused by particles and microorganisms, but also have the potential to cause damage themselves (Rossi, 1986). Macrophages contain a variety of proteases and mediators that are useful in destroying xenobiotics but are destructive to healthy tissue (Brain, 1986). Although recruitment of macrophages to the lung is related to the dose, the adaptive increase in macrophages can be exceeded (Bowden, 1986). This threshold may vary among species. The alteration of macrophage functioning has the potential to shift the balance between protective and adverse effects.

Concurrent with the action of inhaled agents upon critical cell types in the respiratory tract, a portion of the dose in the pulmonary region is likely to be transported across the alveolar epithelium and enter systemic circulation. Changes in permeability can result from the action of some of the mediators and proteases mentioned. The greater the amount reaching systemic circulation, the greater the likelihood for adverse effects in other systems (e.g., liver, kidney, central nervous system). The rapidity and extent to which systemic absorption occurs and the time-to-steady-state blood levels are influenced by (1) ventilation rates and airway mechanics, (2) blood transit time in capillary beds (i.e., perfusion limited), (3) metabolic conversion in the respiratory tract and other organs, (4) alveolar surface area, (5) thickness of the air-blood barrier, and (6) the blood:air and blood:tissue partition coefficients. Many of these factors vary among species and, thus, should be considered in key study identification.

After the inhaled agent enters systemic circulation, the liver may produce additional metabolites that, if the half-life is sufficiently long, may re-enter the lungs and exacerbate the portal-of-entry effects or produce additional adverse effects (Boyd and Statham, 1983). Other agents, that do not require bioactivation, have been shown to damage the lung when applied systemically (Kehrer and Kacew, 1985).

Exhalation of volatile agents (including from administration routes other than inhalation) is an important excretory pathway that is dependent on tissue levels and exposure regimen. For inhalation exposures, the exposure duration influences the amount of chemical entering the systemic circulation, the amount metabolized, and the concentration of the chemical in tissues. Using a simulation model, Fiserova-Bergovera et al. (1984) demonstrated that for chemicals that are not metabolized, tissue concentrations of "poorly soluble" ($\lambda_{oil/gas}$ <10) chemicals change very minimally after two hours of exposure. The pulmonary uptake rate approaches zero at the end of a 2-hour exposure and apparent equilibrium is established. "Easily soluble" chemicals ($10 \geq \lambda_{oil/gas} \leq 10{,}000$) require more than one day of exposure to reach apparent equilibrium and "highly soluble" chemicals ($\lambda_{oil/gas}$ >10,000) require more than 1 year of exposure. If the chemical is metabolized, pulmonary uptake and the amount metabolized increase with exposure duration, but the effect of metabolism may be more complex if exposure concentrations are so high that metabolic pathways approach saturation kinetics and cause metabolism to deviate from first order kinetics.

Conversely, pulmonary clearance decreases with increasing biosolubility (refers to solubility of gases and vapors in biologic materials) and thereby affects the cumulation of chemicals during intermittent exposure regimens. Simulation of an 8 hour/day, 5 days/week schedule for a three-week exposure duration to a 70 kg man showed that poorly soluble chemicals (as defined previously) have no tendency to accumulate in the body, while easily and highly soluble chemicals do have a tendency to accumulate because the intermissions between exposures are not long enough to allow the chemical to be removed from adipose tissue (Fiserova-Bergovera et al., 1984). Excursions in exposure concentrations had a great effect on tissue concentrations of poorly soluble chemicals, but had little effect on tissue concentrations of highly soluble chemicals. Concentrations in well-perfused tissues were more affected by excursions in exposure concentrations than concentrations in muscle or adipose tissues.

The results of these simulation efforts emphasize the uncertainty that the dual function (uptake and exhalation) of the respiratory system adds to any attempt to estimate either respiratory tract or extrarespiratory "dose" of volatile agents. These simulations also emphasize the need for careful consideration of the uptake, metabolism, and excretion parameters for these agents when attempting the exposure duration and concentration conversions discussed in Chapter 4, and when ruling out the possibility of a pulmonary endpoint when using oral data as part of the data base.

3. QUALITATIVE EVALUATION OF THE DATA BASE

The aim of the inhalation RfD methodology is to establish a relationship between a particular agent in the air and a specific health effect. Evidence must be collected from diverse sources and synthesized into an overall judgment of health hazard (Hackney and Linn, 1979). Qualitative evaluation of a diverse data base necessitates a systematic approach for obtaining agreement on the validity and selection of studies to be used in the quantitative methodological procedures of the risk assessment.

3.1 GUIDELINES FOR SELECTIONS OF KEY STUDIES

Key studies are those that contribute most significantly to the weight of evidence as to whether or not a particular chemical is potentially hazardous in humans (U.S. Environmental Protection Agency, 1987a). The studies also may be used in the quantitative dose-response analysis of risk assessment. These studies are of two types: (1) epidemiologic, clinical or case reports on humans; and (2) experimental studies on animals. Each has unique considerations that will be addressed separately. Once the key studies demonstrating the critical toxic effect have been identified, the selection of effect level and the inhalation RfD derivation arises from an objective scientific evaluation of the data available on the chemical. The limitations and the uncertainty factors involved in this derivation are a function of the quality of the key study and will be addressed in Section 3.2. Data base deficiencies and alternative approaches for risk assessment will be presented in Section 3.3.

3.1.1 Human Data

Utilization of human data avoids the necessity of extrapolating from animals to humans, thereby decreasing uncertainty in the risk assessment. Such data have often been useful to the oral RfD work group in qualitatively establishing the presence of an adverse effect in exposed human populations

(U.S. Environmental Protection Agency, 1987a). There are significantly more human data on inhalation than on ingestion exposures, however, so that criteria for evaluating studies and their results need to be stated explicitly. Since 1977, when the Clean Air Act identified goals related to air quality and health, the task of clarifying how population studies can be used for determining scientifically reasonable standards and how to define an adverse respiratory health effect has been rigorously debated (Lebowitz, 1983; American Thoracic Society, 1985; National Research Council, 1985). Many of the results from these efforts can be applied as guidelines for the inhalation RfD methodology.

Three types of human studies are most often utilized to obtain data pertinent to understanding the risk of chemicals to humans: (1) epidemiologic studies, (2) clinical studies or controlled exposure experiments, and (3) case reports (Erdreich and Burnett, 1985). Each of these three study types can provide important information needed to protect public health. When using these studies for risk assessment, several factors are important in evaluating their quality and in determining the level of certainty associated with their use. The factors that are considered when evaluating an epidemiologic study are relevant in evaluating the other types of human studies, but the discussion on epidemiologic studies is the most extensive.

3.1.1.1 Epidemiologic Data. There are essentially three areas of concern in assessing the quality of an epidemiologic study. These involve the design and methodological approaches used for: (1) exposure measures, (2) effect measures, and (3) the control of covariables and confounding variables (Lebowitz, 1983).

The study population and study design must adequately address the health effect in question in order to support a risk assessment (Lebowitz, 1983). In order to accomplish this goal, the exposure measures must be appropriate and of sufficient quality; the statistical analysis methods must be suitable to the study design and goals; the health effect measures must be reliable and valid; and the covariables and confounding variables need to be controlled or eliminated.

3.1.1.1.1 Assessment of exposure measures. The problem of the accuracy and relevance of exposure measurements is not unique to epidemiologic investigations, but it can be exacerbated due to the longterm nature of these studies. For example, the nature of aerometric data changes over time because of different industrial hygiene practices and because individuals change jobs and residences, and thus their exposures change over time. Accurate documentation of air toxicant levels is, therefore, critical in determining the usefulness of an investigation as well as documentation that the analysis of the air toxicant is appropriate and of sufficient sensitivity. It also is advisable to have the concentrations of other pollutants reported to help rule out confounding or interactive effects. The number, location, and timing of monitors must be suitable to allow an appropriate determination of exposure of the subjects to the pollutant being studied and to the pollutants that could confound the results. When appropriate, the exposure measure/estimate should take into account indoor/outdoor exposures and activity and subject location data. The exposure measure/estimate needs to represent the actual exposure in a sufficiently satisfactory way so as to represent the "true" exposure.

3.1.1.1.2 Assessment of effect measures. Effect measures refer to the methods used to ascertain disease indices. For epidemiologic studies these include incidence, standardized mortality ratios, and relative risk ratios.

Criteria for assessment require the proper selection and characterization of both the exposed and control groups. For example, criteria for inclusion in the control category of a case-control study must ensure that this group has no exposure to the agent of concern. Another selection issue is that of needing reference populations or control groups for studies without internal control groups, particularly when evaluating spirometric data (Ferris, 1978; American Thoracic Society, 1979; Crapo et al., 1981; Knudson et al., 1976). Each population used to predict "normal" pulmonary function tests has its own characteristics, which should be considered when used for comparisons. Other considerations include the adequacy of study duration and quality of the follow-up. A disease with a long latency before clinical presentation requires a longer study duration than one with an acute onset. Valid ascertainment (such as verification according to the International Classification of Diseases IX) of the causes of morbidity and death also is necessary.

Evaluation of epidemiologic studies may require interpretation of a variety of subjective health effects data. Questionnaire responses may be biased by the way in which questions are worded, the training of an interviewer, or the setting. A committee of the American Thoracic Society (ATS) charged with defining an adverse respiratory health effect, however, has come to a consensus that "in general, increased prevalence of chronic respiratory symptoms as determined from questionnaire surveys should be considered to be an adverse health effect" (American Thoracic Society, 1985). Questionnaires should be validated as part of the investigation protocol unless a standard questionnaire that has previously been validated is used (Medical Research Council, 1960; Ferris, 1978; National Institute for Occupational Safety and Health, 1986).

In order to assess quantitative results, it is very important to consider differences between statistical significance and medical or biological significance. Both the variability of an outcome measure and the magnitude of an exposure's effect determine the level of statistical significance. For example, data from a large study population analyzed with sophisticated techniques may yield statistically significant effects of small magnitude that cannot readily be interpreted biologically. Conversely, large effects of clinical importance may not be statistically significant if the study population is too small; that is, if the studies presented negative or no-effect results due to the lack of power or the small number of subjects in the study. Judgments concerning medical or biological significance should be based on the magnitude of effect. For example, cough and/or phlegm production can be considered less important than effects resulting in hospital admissions. Underlying assumptions and nuances of the statistical procedures applied to the data also need to be considered. This will probably best be accomplished on a case-by-case basis, as has been done by the oral RfD work group.

Because the inhalation RfD considers both portal-of-entry and systemic effects, it would be helpful to define an "adverse respiratory health effect." An ATS committee published guidelines that defined such an effect as medically significant physiologic or pathologic changes generally evidenced by one or more of the following (American Thoracic Society, 1985):

- Interference with the normal activity of the affected person or persons
- Episodic respiratory illness
- Incapacitating illness
- Permanent respiratory injury or
- Progressive respiratory dysfunction

Appendix C provides detailed descriptions of adverse respiratory effects in humans.

3.1.1.1.3 Assessing the control of confounding and covariables. Epidemiologic investigations have to relate an exposure to a given health effect, but this includes accounting for the "background" health effect (pathologic condition) that exists in individuals due to predisposing factors and pre-existing health conditions, or from other variables, such as occupational exposures.

Various host factors contribute as risk factors for disease and can influence the health indices assessed. For example, asthmatics may be particularly susceptible to effects from exposure to irritant gases. Epidemiologic evaluation of these factors often not only accounts for such interactions but also can help to characterize susceptible or sensitive groups. Covariables can be as important as the major aerometric variables themselves in affecting human health. Other exposures, such as concomitant occupational exposures and smoking, in particular, can affect the disease outcome. Meteorologic variables such as air velocity, temperature, and humidity also are very important factors when considering respiratory health effects. These covariables should be controlled by both the study design and analysis as appropriate.

Assessment of individual epidemiologic studies should bear in mind that the final step in the inferential process from an epidemiologic investigation requires the extension of its results to persons, populations, or settings not specifically included in the study. The confidence with which this is done for positive results is usually based implicitly on how successful the investigators have been in identifying and handling the potential risk factors and covariables that produce or influence the pollution-effect association they have observed. Uncertainties also arise because the general population includes some people, such as children, who may be more susceptible than people in the sample from which the epidemiologic data were derived. Factors such as the "healthy worker" effect and the bias of a predominantly male worker sample must be considered when using occupational studies (National Research Council, 1985). Intraindividual variability concerns are addressed in Section 3.1.1.3.

3.1.1.1.4 Summary. Specific recommendations for the evaluation of epidemiologic investigations have been adapted from Lebowitz (1983), American Thoracic Society (1985), and Interagency Regulatory Liaison Group (1981). Appendix D provides guidelines for evaluating individual epidemiologic studies and the considerations involved in evaluating the statistical analyses.

3.1.1.2 Nonepidemiologic Data. Human data also include clinical studies and case reports. The case reports and acute exposures provide support for the weight-of-evidence decision, but are often of limited utility in establishing a quantitative relationship between environmental exposures and anticipated effects (U.S. Environmental Protection Agency, 1987a). They are often valuable in determining the nature of the effect in humans.

3.1.1.2.1 Clinical studies. Clinical studies may contain exposure-response information that can be used in estimating effects. Most clinical studies combine the strong point of animal toxicology, rigorous control of the experimental exposure and subject, with the strong point of epidemiology, the unquestioned relevance to human health (Hackney and Linn, 1983). In addition, clinical studies can be independently replicated somewhat more easily (requiring a reasonably short time and resource commitment) than the other types. There are limitations, however, that include short exposure duration, "noninvasive" techniques that might not ascertain the full array of effects, and small groups of test subjects. The test atmospheres are usually within that expected to produce only mild and temporary health effects. Certainly, clinical studies should be recognized and given credence to the extent that they are scientifically rigorous, relevant to human health concerns, and can be independently replicated. They may be particularly useful for less-than-lifetime risk assessment. The prediction of long-term effects from short-term observations remains questionable, but confidence in clinical findings can be bolstered by supporting evidence from epidemiology and animal toxicology, and vice versa.

3.1.1.2.2 Case reports. Individual case reports of adverse effects due to a specific agent also can provide some help in evaluating the potential risk from exposure to a toxic air pollutant. These reports are especially valuable qualitatively for indicating that the quantitative effect observed in animals occurs in exposed humans. These reports must be examined carefully and used with discretion since they represent a very small sample and are usually related to heavy exposures (Goldstein, 1983). Nevertheless, these observations should not be overlooked, especially when a large number of case histories exist with the same endpoint. Research needs to address the interrelationships of findings from short-term observations, epidemiology, and animal toxicology, and to establish appropriate links among them in order to support regulatory decisions.

3.1.1.3 Intraspecies Variability and Identifying Sensitive Subgroups. In order to control factors other than the chemical being tested, animals used in toxicity studies (e.g., rodents) are often bred for homogeneity. In contrast, the human population is heterogeneous. The broad genetic variation of the human population in metabolism and in tissue response to chemicals causes individual differences in susceptibility to toxic chemicals. A sensitive or hypersusceptible individual is one who will experience an adverse health effect to one or more pollutants significantly earlier in the course of exposure, or at lower doses than the average individual, because of host factors that predispose the individual to the harmful effects. Sensitive individuals may be those whose genetic makeup puts them at the extreme end of a continuous distribution of a biological function, such as the amount of enzyme production, or those who possess a unique genetic difference, such as an altered enzyme, that makes them markedly different from the general population.

In addition to genetic factors, personal characteristics such as age, sex, health status, or habits make some people more susceptible (Calabrese, 1978). The activity pattern of people is a major host factor influencing the dose-response by its effect on delivered dose. Generally, exercise increases the delivered dose and alters the regional deposition of the dose. The principles involved have not been quantified sufficiently to date, but should be considered qualitatively when comparing studies or population subgroups.

Environmental risk assessment should consider host factors that both increase susceptibility and that occur relatively frequently in the population. Erdreich and Sonich Mullin (1984) estimated the prevalence of population subgroups of individuals who are potentially hypersusceptible to some common pollutants. Table 3-1 shows five categories of individuals who, based on empirical observations or compromised physiological functions, are assumed hypersusceptible to the listed chemicals.

As a result of epidemiologic investigations, it is well recognized that a population of adult workers experiences less morbidity and mortality than the general population (Fox and Collier, 1976; Wen et al., 1983; Monson, 1986). However, sufficient qualitative and quantitative information on interindividual variability and hypersusceptibility for specific chemicals rarely exists.

If the decisions on the RfD are to be made on data derived from subgroups of the general population such as workers who are generally a selected group of healthy adults, the extrapolation procedure must contain appropriate adjustments to account for the anticipated broader variability in the general population. Worker populations are nonrepresentative in terms of age distribution

TABLE 3-1. PREVALENCE OF SUBGROUPS HYPERSUSCEPTIBLE TO EFFECTS OF COMMON POLLUTANTS[a]

Hyper-susceptible	Prevalence[b]	Chemicals[c]	Reference[c]
Embryo, fetus, neonate	pregnant women: 21/1000[d]	carcinogens, solvents, CO, mercury, lead, PCBs, pesticides	Rice, 1981; Kurzel and Cetrulo, 1981; Saxena et al., 1981
Young children	ages 1-4: 70/1000	hepatotoxins, PCBs, metals	Calabrese, 1981; Friberg et al., 1979
Lung disease	emphysema, asthma: 37/1000[e]	ozone, Cd, particulates, SO_2, NO_2	Holland et al., 1979; Redmond, 1981
Coronary heart disease	coronary heart disease: 16-27/1000[e]	chlorinated solvents, fluorocarbons, CO	McCauley and Bull, 1980; Aviado, 1978 U.S. Environmental Protection Agency, 1984a,b
Liver disease	liver abnormalities: 20/1000[f]	carbon tetrachloride, PCBs, insecticides, carcinogens	Calabrese, 1978

[a] Source: Adapted from Erdreich and Sonich Mullin, 1984.

[b] All estimates based on 1970 census.

[c] Representative samples of chemicals to which these individuals may be hypersusceptible. Some evidence from animal studies only.

[d] Authors' estimate from 1970 census statistics data.

[e] Health Interview Survey (National Center for Health Statistics, 1970).

[f] Health Interview Survey (National Center for Health Statistics, 1975).

and general health status. Hypersusceptible people may not be represented because they may not seek or sustain employment, particularly in situations such as those represented in workplace exposure studies. Occasionally, data are available on more sensitive subgroups such as children or asthmatics. In these cases, risk assessments can be made for the general population with greater confidence. In the absence of data on the more susceptible individuals in the population or lack of identification of such individuals, uncertainty factors are used to protect unidentified individuals at greater risk.

There are two steps necessary to obtain information addressing the problem of sensitive individuals: (1) examine chemical-specific data for empirical evidence of sensitivity and hypersusceptibility, and (2) ascertain whether the mechanism of toxicity for a given chemical suggests that any population group would be extremely sensitive.

In addition to this chemical-specific evaluation, guidance should be developed concerning the prevalence of sensitive subgroups and the range of sensitivities in the general population exposed to inhaled toxicants. The U.S. Environmental Protection Agency (1986a) has initiated research to assess the magnitude of interindividual variability in pharmacokinetic parameters related to the delivery of the biologically effective dose, in order to develop guidance for appropriate uncertainty factors. Differences among normal healthy adults may be as much as 10-fold (U.S. Environmental Protection Agency, 1986a). Therefore, the potential that exists for broad differences when children, the elderly, the ill, and those previously exposed are included must be considered.

The issues discussed in this section are summarized as follows:

Evaluation of the Epidemiologic Data Base

- Examine epidemiologic and clinical data for dose-response information in potential or previously identified sensitive groups (e.g., studies in asthmatics, children).
- Examine animal data for studies in models of sensitive individuals.
- Evaluate epidemiologic studies to ascertain genetic and personal factors that increase the risk of adverse response. Evaluate implications of these risk factors for identifying sensitive groups.
- Examine data for reports of ranges of responses or response variables, and for data containing individual responses. This is particularly important in evaluating human data for assessing the range of variability in response because epidemiologic studies may not include exposure levels associated with a NOEL, but with an effect.
- Evaluate available biological monitoring data and clinical and experimental data for indications of characteristics of increased susceptibility. For example, respiratory irritants may induce responses earlier in individuals with α-1-antitrypsin deficiency.
- Evaluate data on mechanisms of toxicity, pharmacokinetics, and critical target organs to identify characteristics that may imply broad interindividual variability or hypersusceptible individuals. For example, the elderly may be more sensitive to certain chemicals in relation to age-related changes in oxidative metabolism potential.

Evaluation of Individual Studies

- Assess the makeup of the study population and control groups to identify presence or absence of sensitive individuals. Data on healthy workers, for example, are not representative of the general population and will require reduction of NOAELS or LELs by uncertainty factors.
- Consider the activity pattern of the subjects. Whether the subjects received exposure while at rest or at level(s) of exercise will influence the inhaled dose as well as the pattern of deposition.
- In longitudinal (cohort) studies, evaluate information in relation to the natural history of the disease, i.e., the progression of lesions. Normal changes over time, such as increased FEV_1 as children get older, and decline of FEV_1 with aging in older adults, should not be adversely affected. Cross-sectional studies may suggest such associations but will not support causality as strongly as will cohort studies.
- For parameters that have known variability with age, such as FEV_1, evaluate results within age groups and ascertain whether appropriate reference populations were used.

Areas for further investigation and development of specific guidance include:

- To what extent can we develop guidance on which conditions and diseases predispose individuals to hypersusceptibility? It is important to emphasize conditions that are more common in the population (3-5%). Susceptibility factors can be linked with characteristics of chemicals or to specific chemical classes to facilitate generic risk assessment procedures.
- How do known differences in components of respiratory function, such as age-related differences in FEV_1, affect susceptibility to systemic toxicity from airborne chemicals?

3.1.2 Animal Data

When the data base lacks appropriate information on effects in humans, as is frequently the case, the principal studies are drawn from experiments conducted on nonhuman mammals. Animals most often used include the rat, mouse, guinea pig, hamster, rabbit, monkey, and dog. Such animal studies have often been conducted with controlled exposure conditions on relatively homogenous populations, but nevertheless, present the risk assessor with concerns about evaluating dose and exposure regimen. Unlike the human, the laboratory rodent strains, because of inbreeding, have homogeneous constitutions. Genetic background differences and numerous other interspecies differences are confounding factors during key study selection.

Evaluation of the quality of individual animal toxicity studies requires consideration of factors associated with the study's hypothesis, design, execution, analysis, and interpretation (U.S. Environmental Protection Agency, 1987a). Guidelines for assessing individual animal studies are provided in Appendix E and are adopted from a number of recommendations (National Research Council, 1984; Society of Toxicology, 1982; James, 1985; Muller et al., 1984; Lu, 1985a). The reader is referred to this appendix for a more detailed description of those issues discussed here.

3.1.2.1 Appropriateness of Species as a Model for Humans. Identification of the most appropriate animal species is the end result of an interpretative process that examines all facets of a data base from study design to data relevance to the extrapolation methodology.

The most sensitive species is selected from evaluation of key studies. While this approach (i.e., NOAEL identification) may have the advantage of affording a greater degree of protection, the species most sensitive to an agent may not be as toxicologically relevant as other species for extrapolation to man because of a variety of interspecies variables.

Selection of an appropriate animal model and key study depends on the depth of understanding of the human disease syndrome, adverse effect, or indicator of toxicity selected as the criterion for evaluation. While a particular animal species may share a number of similarities with humans in respiratory tract physiology, it may be dissimilar in crucial parameters and thus, make it a less than adequate source as a model. This subject area has been reviewed recently (Hakkinen and Witschi, 1985) and various mammalian species (rat, hamster, rabbit) were identified as appropriate species for extrapolation from several perspectives. Other reviews that discuss the current limitations and need for the development of animal models as surrogates for humans include those of Reid (1980), Slauson and Hahn (1980), and Calabrese (1983).

For agents whose toxicological outcome is dependent on the degree to which it is metabolized, the most appropriate animal species is contingent upon proper evaluation of the numerous interspecies differences with respect to metabolism (see also Section 2.2). The studies of Plopper et al. (1983) suggest that animal species differ widely in metabolizing potential of the respiratory tract. Hamsters and rabbits have much greater metabolizing potentials than do monkeys and rats. Interspecies differences in the metabolic pathway, as shown for xylene (National Toxicology Program, 1986), may serve as a basis for selecting one study for RfD derivation and rejecting another.

Appropriate animal model selection may be contingent upon pathological identification of early changes consistent with the human syndrome; for example, a clear choice of an appropriate animal species has not been established for emphysema (Snider et al., 1986). The hamster may be considered as most similar to man, with respect to emphysema, as measured by serum α-antitrypsin levels. Hamsters have the lowest antiprotease levels of 10 species tested (Snider et al., 1986). Individuals with deficient blood levels because of a genetic defect are characterized as a high-risk subgroup for emphysema. However, primates have comparable antitrypsin profiles (Ihrig et al., 1971).

Species-dependent variables in mucous production and secretion are factors in selecting an appropriate animal model (see also Section 2.2). Ozone exposure, for example, increases mucous secretion in rats but not in monkeys (Gardner, 1984).

3.1.2.2 Study Design. An ideal study addresses a clearly defined hypothesis, follows a carefully prescribed protocol, is conducted in adherence to good laboratory practice, and includes appropriate and sufficient subsequent analysis to support its conclusions. The U.S. EPA Good Laboratory Practice Standards (Code of Federal Regulations, 1983a,b) are designed to ensure the quality and integrity of data used in hazard evaluation. These regulations contain detailed guidance on provisions for personnel, facilities for animal care, animal supply, handling of test and control substances, equipment, operation of testing facilities, characterization of test and control chemicals, protocol and conduct of a laboratory study, report records, record storage, and record retrieval. Studies that do not precisely follow these guidelines may still be judged adequate if the committee to develop inhalation RfDs determines that, in the context of results, the deviations are not important. The type of deviation (variation) and its magnitude, as well as the potential for its interaction among all the variables, must be assessed by the committee (National Research Council, 1984). For example, a study may still be judged adequate, despite an insufficient number of test animals specified by the appropriate reference protocol guidelines, if the results are so definitive that the addition of more test animals would almost certainly not have affected the conclusion. Risk assessments that use studies with deficiencies may include a modifying factor to account for the added uncertainty in its use (see Section 4.1.).

The appropriate application of statistics in both the design and interpretation of studies is an area in animal toxicity testing that is often neglected or distorted (Muller et al., 1984). Consideration of statistical applications restricted to confirmatory analysis (i.e., outcome is dependent on the mathematically randomized test condition and is independent of other observations) vs. exploratory analysis (i.e, many tests on a variable) should be emphasized.

3.1.2.3 Study Validity and Relevance to Extrapolation. The validity of the study and its relevance to human extrapolation is another major area to consider when assessing individual animal studies. It involves the evaluation of a number of factors, including all elements of exposure definition (dose, duration, administration route, and physicochemical characterization of the chemical used), reliability of and limits to the procedures used for both exposure and effects measurements, relevance of the dose level tested to the anticipated human exposure level, nature of the effect (consistency with the area of toxicology assessed and the suspected mechanism of action), and the similarities and differences between the test species and humans (e.g., in absorption and metabolism).

Animal studies are conducted using a variety of exposure scenarios in which the magnitude, frequency, and duration of exposure may vary considerably. Studies may use different durations (acute, subchronic, and chronic) as well as schedules (single, intermittent, and continuous). All of these studies contribute to the hazard identification of the risk assessment. Special consideration should be addressed to those studies of appropriate duration for the reference level to be determined (i.e., chronic investigations for the RfD).

These exposure concerns (dose and duration) are compounded when the risk assessor is presented with data from several animal studies. An attempt to identify the animal model most relevant to humans should be made on the most defensible biological rationale (e.g., comparable metabolism and pharmacokinetic profiles). In the absence of such a model, the most sensitive species (i.e., the species showing a toxic effect at the lowest administered dose) is adopted for use as a matter of science policy at the U.S. Environmental Protection Agency (1987a). This selection process is more difficult if the animal data are for various exposure routes, especially if the routes are different from that in the human situation of concern.

Because the data base may be deficient for the route of exposure of interest, it is the Agency's view that the toxicity potential manifested by one route is relevant to any other exposure route unless convincing contrary evidence exists (U.S. Environmental Protection Agency, 1987a). Consideration must be given to the differences in the pharmacokinetics for the chemical resulting from the different exposure routes. Bioavailability of the chemical administered is another important factor for consideration/uncertainty in the evaluation of dose. Detailed consideration is given to this topic in Section 4.1.1.2.

3.1.3 Summarizing the Evidence

The culmination of the hazard identification phase of any risk assessment involves integrating a diverse data collection into a cohesive, biologically plausible toxicity "picture"; that is, to develop the weight-of-evidence that the chemical poses a hazard to humans. The salient points from each of the animal and human studies in the entire data base should be summarized as should the analysis devoted to examining the variation or consistency among factors (usually related to the mechanism of action), in order to establish the likely outcome for exposure to this chemical. From this analysis, an appropriate animal model or additional factors pertinent to human extrapolation may be identified.

The utility of a given study is often related to the nature and quality of the other available data (Erdreich and Burnett, 1985). For example, clinical descriptions can provide insight on pharmacokinetics and may validate that the target organ or disease in animals is likely to be the same effect observed in the exposed human population. However, if a cohort study describing the nature of the dose-response relationship were available, the clinical description would rarely give additional information. An apparent conflict may arise in the analysis when an association is observed in toxicologic but not epidemiologic data, or vice versa. The analysis then should focus on reasons for the apparent difference in order to resolve the assessment. For example, the epidemiologic data may have contained other exposures not accounted for, or the animal species tested may have been inappropriate for the mechanism of action. A framework for approaching data summary is provided in Table 3-2. Table 3-3 provides the specific uses of various types of epidemiologic data in such an approach. These guidelines have evolved from criteria used to establish causal significance, such as those developed by the American Thoracic Society (1985) to assess the causal significance of an air toxicant and a health effect. The criteria for establishing causal significance can be found in Appendix F. In general, the following factors enhance the weight-of-evidence on a chemical (U.S. Environmental Protection Agency, 1987a):

- Clear evidence of a dose-response relationship
- Similar effects across sex, strain, species, exposure routes, or in multiple experiments
- Biologically plausible relationship between metabolism data, the postulated mechanism of action, and the effect of concern
- Similar toxicity exhibited by structurally related compounds,
- Some correlation between the observed chemical toxicity and human evidence

Developing improved weight-of-evidence schemes for various noncancer health effect categories is the focus of ongoing efforts by the Agency to improve health risk assessment methodologies (Perlin and McCormack, 1988).

The greater the weight of evidence, the greater the confidence in the conclusion derived. Another difficulty encountered in this process is when certain studies produce clearly positive or negative results, yet may have to be considered as flawed. The flaws may have arisen from inappropriate design or execution in performance (i.e., lack of statistical power or adjustment of dosage during the course of the study to avoid undesirable toxic effects). The treatment of flawed results is critical; although there is something to be learned from every study, the extent that a study should be used is dependent on the nature of the flaw (Society of Toxicology, 1982). A seriously flawed negative study could only provide a false sense of security, whereas a flawed positive study may be entitled to some weight. Although there is no substitute for good science, grey areas such as this are ultimately a matter of scientific judgment. The risk assessor will have to decide what is and is not useful within the framework outlined earlier.

TABLE 3-2. PROPOSED APPROACH FOR SUMMARIZING THE EVIDENCE FROM DIVERSE DATA

CONCEPT 1: STRENGTH OF THE ASSOCIATION

The stronger the association, the greater the confidence that the agent causes the effect.

- Presence of low LD_{50}, low NOEL, high potency index
- Dose-response gradient evident
- High incidence rate, large excess risk
- High level of statistical significance in relevant studies

CONCEPT 2: CONSISTENCY

The association is observed in various circumstances.

- Observed in a number of experimental species
- Various routes
- Different dose regimens
- Descriptive epidemiologic data
- Analytical epidemiologic studies

CONCEPT 3: BIOLOGICAL PLAUSIBILITY

The association is plausible in terms of other scientific information related to the causal mechanism.

- A gradient of responses observed
- Short-term or *in vitro* tests
- Pharmacokinetics
- Molecular action and pathology
- Structure-activity relationship
- Preclinical indicators
- Biological monitoring of exposure

Source: Erdreich, 1988.

Studies meeting the criteria detailed in Sections 1.1 and 1.2 (epidemiologic, nonepidemiologic and/or experimental studies on animals that "fit" into this framework) are used in the risk assessment phase.

3.2 TOXICOLOGICAL ISSUES IN DATA EVALUATION

3.2.1 Qualitative Evaluation of Dose Response and Dose Effect Data

3.2.1.1 Relationship to the Uncertainty Factor Approach. Evaluation of dose-consequence relationships involves two distinct steps. The first relates to the evaluation of an individual study with emphasis on the following:

TABLE 3-3. HUMAN DATA FOR USE IN HEALTH RISK ASSESSMENT

Study (Alternative Terms)	Comment on Potential Use
	EPIDEMIOLOGIC DATA
Cohort (longitudinal, prospective, incidence)	Rates as percent response useful in risk characterization. Measure of excess risk can be obtained. If dose or exposure data are available, dose-response curves can be constructed. Studies with ordinal exposure data support strength of evidence and hazard identification.
Case-control (retrospective, dose or case-referent)	No direct measure of disease rates. If exposure data are available, a NOEL may be identified.[a] Studies with ordinal or nominal exposure data may support strength of evidence and hazard identification.
Cross-sectional (prevalence)[b]	Similar to case-control for short-term effects. Prevalence data less reliable for effects from chronic exposures.
Geographic correlation[b]	An inexpensive screening procedure. Crude indicator of potential hazard. Rates are usually only indirectly related to exposure. Generates hypotheses for analytical studies.
Clinical trials	Generally not applicable to environmental issues, because exposures are treatments or preventive measures. Intervention trials in which an exposure is removed or changed (e.g., medication, smoking, diet) are useful in strength of the evidence for evaluating causality.
	NONEPIDEMIOLOGIC DATA
Experimental studies	The only human data with controlled exposure levels. Usually interval level exposure data but low dose, limited exposure time. Use for hazard identification, dose-response, risk characterization.
"Exposed-control" comparisons (noncohort; see text for discussion)	Rates may be biased because of self-selection or incomplete ascertainment of exposed population. Cannot be used to support absence of hazard. Clinical descriptions useful for hazard identification.

(continued on the following page)

TABLE 3-3. (continued)

Study (Alternative Terms)	Comment on Potential Use
Case series[d]	Can be used to demonstrate hazard if syndrome is unusual. Usually high level, short-term exposure. May yield data point for adverse-effect levels. Cannot be used to show absence of hazard.
Case reports	Suggests nature of acute endpoints in humans. Cannot be used to support absence of hazard.

Source: Adapated from Erdreich and Burnett, 1985.

[a] Exposure history is difficult to reconstruct, particularly outside of the occupational setting.

[b] May be available pertinent to air pollution exposure.

[c] Several cases seen by or reported by a single investigator. Cases may be attributed to unique exposure incident, but total exposed population is not defined.

- Identifying the critical effect. The critical effect has been defined as the effect that occurs first on the increasing dose scale. The critical effect is either an adverse effect or a known precursor to an adverse effect (U.S. Environmental Protection Agency, 1987a). The American Thoracic Society has proposed a classification scheme for severity of respiratory effects in humans which is presented in Appendix C.

- Evaluating the dose-response curve for the critical effect with the goal of identifying doses that bracket the experimental threshold region.

These issues are selected based on the assumption that the study has already been evaluated for adequacy in terms of design and conduct. Issues pertaining to the evaluation of inhalation studies are discussed in Chapters 2 and 4.

The second step involves comparison of dose-response and dose-effect curves across studies (within and across species). The first comparison is a qualitative evaluation of effects. When disparity in dose-effect patterns is apparent, studies need to be evaluated to ascertain, if possible, whether the differences are due to differences in the monitored endpoints or procedure across studies, or whether they suggest that species differences exist in dose-effect curves (see Section 4.1).

If species differences are apparent, the question arises as to which species is the most appropriate model for humans. Differences in dose-effect curves could be due to inherent differences in target receptor sensitivity (pharmacodynamics) or to differences in concentration of the compound or metabolite reaching the receptor (pharmacokinetics). This distinction is important when trying to identify the most appropriate species for modeling the human response.

The dose delivered to the target tissue is important when evaluating dose-effect and dose-response curves across species. The target tissue dose is determined by absorption, distribution, metabolism, and excretion. For the inhalation route, the absorption component is particularly problematic. Although absorbed doses per se have not been estimated as part of the RfD process, the assumption has been made implicitly that absorption is either equivalent across species, or that the divergences are minimal and can be subsumed within the interspecies uncertainty factor along with other pharmacokinetic and pharmacodynamic considerations. For inhalation, not only is there a question of absorption estimates, but there also is uncertainty in estimating the amount of material inhaled and/or deposited and, thus, available for absorption, as well as potential differences in uptake of material from the pulmonary tract due to the wide differences across species in airway anatomy and physiology and body fat compartments (see Section 2.1). These differences suggest that until more sophisticated methods of estimating "equivalent" inhalation doses across species are developed, estimation of equivalent dose, as one subpart of the interspecies extrapolation question, may be more uncertain than for oral exposures. Procedures applicable to relatively insoluble particles for adjusting doses based upon described differences in deposition across species are discussed in Chapter 4. Where appropriate, adjustments in doses based upon known interspecies differences in pulmonary deposition must be applied before arraying the dose effect data to compare species sensitivity. Approaches for estimating interspecies dose differences for gases and vapors of organic solvents which are metabolized have been developed (Fiserova-Bergerova, 1983) using physiologically-based pharmacokinetic models. This type of model has been applied by EPA for quantitative cancer risk assessment for perchloroethylene and methylene chloride (U.S. Environmental Protection Agency, 1986e, 1987b), but general applicability is not yet possible due to the need for chemical- and species-specific information on metabolism which is not available for most chemicals. Further validation of these models and development of the necessary data base should result in a routinely applicable approach to interspecies dose adjustments. Equivalent approaches for dose adjustment for soluble gases and hygroscopic particles are not yet as fully developed. Error in estimation of equivalent dose also may complicate selection of the most appropriate animal model for extrapolation. In particular, difficulties may be encountered when human studies with inadequate exposure information suggest effects that differ from the animal models, or when human data are absent and the critical effect in animals has no known human counterpart.

The final stage in the data evaluation process is the accurate estimation of a subthreshold exposure level for the heterogeneous human population. Although it would be easy to project "safe" doses for many compounds which are orders of magnitude below actual threshold doses with a great deal of confidence, achieving these minimal exposure levels could be very costly and/or technologically infeasible. Therefore, the goal is to accurately project a subthreshold dose that is close to the threshold. If we could precisely characterize the human dose-response curve for the known human critical effect while completely characterizing human variability, then there would be little uncertainty in these RfD estimates. The current RfD process is geared to develop subthreshold estimates in the presence of uncertainty. For example, if a range of species sensitivities is apparent (following dose correction as described in Chapter 4) and human data are unavailable, it is assumed that the most sensitive species should be used to develop an RfD. When chronic data are unavailable, subchronic data are adjusted by an empirical factor when, in some instances, there may not be a progressive dose-time interaction. As a result, with the elimination of uncertainty many of the determined subthreshold doses could potentially be higher or lower than those presently proposed.

The uncertainty factor approach addresses major areas of uncertainty relating to the inability to know the collective human dose-response curves for the critical effect. These factors are empirically based. Their initial proposal and implementation have been restricted to oral exposures. Validation of these factors based upon experimental data has been attempted, but is difficult primarily due to deficiencies in the available data base. If this empirical factor approach is applied to the inhalation RfD process, a critical question becomes whether or not any component(s) of the extrapolation process leading to the RfD estimate appears to be inherently more uncertain or variable for the inhalation route. Particular aspects of this question will be discussed in later sections of this document. Specific information relevant to uncertainty factors per se is presented in Chapter 4.

3.2.2 Selecting Effect Levels: Inhalation-Specific Issues

Traditionally, ADI levels have been calculated by dividing the appropriate effect or no-effect level of the critical toxic effect from human or animal toxicity studies by one or more uncertainty factors. The critical effect is defined as either the adverse effect* that first appears in the dose scale as dose is increased, or as the known precursor to the first adverse effect. It is assumed that if the critical effect is prevented, then all subsequent adverse effects are prevented. The derivation of the RfD follows these same principles. Henceforth, the term RfD will be used.

As is often the case, NOELS, NOAELs, and LOAELs exist in a given data base for several animal species. When comparing effect levels across species, it is assumed that the doses will be adjusted to reflect currently characterized interspecies differences in pulmonary deposition (see Chapter 4). What is the appropriate choice of no-effect or effect level given this diversity? In the course of verification discussions on various RfDs during the last year, the oral RfD work group has provided some common ground on this issue. The work group suggested the following conditions in choosing the appropriate animal effect or no-effect level as a basis of an RfD:

- When all scientific issues and effect or no-effect levels are generally equal, choose the most appropriate effect level of a species that is known to resemble the human in response to this particular chemical, for example, by similar toxicokinetics.
- When the previous condition is not met, choose the most sensitive species as judged by an interspecies comparison of the highest individual species NOAEL (or NOAEL) and its LOAEL (or LEL).

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*Here adverse effects are considered to be functional impairments or pathological lesions that may affect the performance of the whole organism, or that reduce an organism's ability to respond to an additional challenge (Federal Register, 1980). One of the major problems encountered with this concept is the reporting of "observed effect levels" as contrasted to "observed adverse effect levels." The terms "adverse" and "not adverse" are at times satisfactorily defined, but more subtle responses are being identified because of increasingly sophisticated testing protocols, resulting in a need for judgment regarding the exact definition of adversity.

- If scientific issues or effect or no-effect levels are judged to be generally equal, choose the effect or no-effect level that yields the RfD with greatest confidence reflecting quality of the study and data base.

An expanded discussion and an example exercise of choosing the effect level is provided in Appendix G.

In order to implement the guidance as described, adverse and nonadverse effects must be distinguished. Historically, the distinction between adverse effects and nonadverse effects has been problematic. Although numerous groups have addressed this issue, most often conclusions contain an element of scientific judgment in addition to objective criteria. Considerable experience and precedent for such decisions have accrued over the last several years in the process of developing oral RfDs and other health-related guidelines. Although inhalation data have in some instances been utilized for the development of oral estimates, the information content of the studies in terms of respiratory system effects has not been rigorously evaluated, because it was appropriately not considered relevant to the oral guideline efforts. As a result, the question of adversity for pulmonary endpoints has not been extensively explored in the context of oral RfD development. However, other groups have addressed this and consensus guidelines have been developed. The American Thoracic Society committee report has been discussed previously and is reproduced in Appendix C.

There still appears to be considerable uncertainty concerning how to differentiate in the early stages of respiratory disease between acute reversible effects, which are the immediate consequence of an exposure episode, and potential progression to chronic, nonreversible pulmonary pathology. This is an important issue both in terms of evaluation of pulmonary effects per se, as well as for decisions concerning the critical effect in inhalation studies.

For inhalation studies in particular, there is a dichotomy in terms of the types of endpoints monitored in human versus animal studies. Human data concerning the consequences of inhalation exposure generally consist of information on subjective symptoms along with clinical data concerning pulmonary function. The relationship between the clinical picture and lung pathology is poorly defined. On the other hand, animal standard toxicological protocols generally incorporate pulmonary tissue evaluation as part of the routine necropsy, but do not evaluate pulmonary function. Of course, once the lung has been identified as a target tissue, more detailed studies of it as a target organ may be conducted. When these more detailed data are available, two additional questions are raised: (1) how do we evaluate the significance of alterations in test species' pulmonary performance in terms of potential human effects and, (2) if tests showing differences in pulmonary biochemistry are available, what is the utility of the biochemical changes as predictors of disease? Correlations between functional decrements and immunologic, biochemical, and pathologic changes need to be quantitated. Work in progress on animal models (see Section 3.1.2.1), biological exposure indices (Lowry, 1986), and *in vitro* alterations of lung biochemistry as predictive of lung disease (Last, 1983) will contribute to this end.

For present purposes, each inhalation study should be evaluated for possible indications that the respiratory system is the critical target organ. Animal data that provide only cursory evaluation of pulmonary endpoints make careful evaluation of human studies essential. Human data should be carefully evaluated with special emphasis on the significance of respiratory system endpoints. In instances where extrarespiratory effects are the critical effect, effect levels would be evaluated in a manner consistent with decisions made in the oral RfD process. This approach was initially described in Federal Register (1980). Existing, verified RfD cover sheets provide insight into current judgments concerning adversity of particular endpoints. Extrapolation from oral to inhalation exposures may be utilized only after careful consideration of factors presented in Sections 4.1.1.3 and 4.3.

For compounds that appear to produce their critical effect within the respiratory system itself, decisions concerning adversity need to be made on a case-by-case basis. Appendix C provides specific information concerning evaluation of the severity of pulmonary endpoints in humans. Costa and Tepper (1988) provide an excellent summary of lung function assessment in animals.

Although most pollutants would be expected to elicit a dose-response upon exposure, some pollutants cause tolerance/adaptation and some are atypical, such as those that act by allergic or asthmatic mechanisms. These allergic sensitizers may be considered a subgroup under agents that produce their critical effect in the respiratory system. Toluene diisocyanate is a well-known example of a sensitizing agent that affects immunological and pharmacological mechanisms and induces asthma. Sensitizing responses appear to be triggered by high initial doses. Subsequently, any level of exposure may be sufficient to induce the asthmatic syndrome in sensitized individuals. There is evidence that IgE antibody levels and inflammatory pulmonary reactions play a role in such syndromes. If these are indeed nonthreshold phenomena upon challenge exposure, then methods other than the traditional uncertainty factor approach will be required to address this subclass of compounds for quantitative risk assessment.

Areas for further investigation and development of specific guidance include the following:

- Specific guidance for evaluation of pulmonary endpoints in terms of adversity/severity for both human data and animal investigations.
- Specific guidance for interpreting effects when both human and animal data are available.
- Specific guidance for interpreting the impact of short-term exposures to human subjects and subsequent pulmonary effects to chronic exposure situations, if any.
- Specific guidance concerning the comparability of effect levels following intermittent exposures to continuous exposure scenarios.
- Specific guidance on how to deal with sensitizing agents in the RfD process.

3.3 DEFICIENT DATA BASES AND ALTERNATIVE SOLUTIONS

The assessment of the total toxicological data base available for the chemical at that time must be evaluated to derive an RfD (Clegg, 1979). In addition to the uncertainties discussed in Section 3.2, determination of an RfD also involves a judgment about the study used in the RfD calculation. These judgments relate to quality and completeness of the entire data base, including uncertainty in the dose-response information and the estimated NOEL. Although there is no readily definable way to measure the magnitude of uncertainty in any given RfD (Environ Corporation, 1985) at present, research to address this issue is underway. The minimum data needs for establishing an RfD predicated on addressing this uncertainty are discussed in Section 4.1.1.1. Section 3.3.2 discusses the role of occupational exposure limit values in RfD development.

3.3.1 Guidance on Evaluating a Data Base for Completeness

Current toxicity testing strategies are hierarchical sequences of tests designed to develop a profile of a chemical's toxicity (Environ Corporation, 1985). Initial testing tiers consist of relatively rapid, inexpensive tests designed to identify acute toxicity. This information is not directly useful in predicting chronic adverse effects in humans, but can be used to guide decisions as to type and extent of continued testing, such as subchronic, chronic or reproductive bioassays.

The toxicity "profiles" or information required as a minimum data base also are somewhat structured according to this hierarchy. The magnitude of insufficiency varies on a case-by-case basis and is reflected in the rating of uncertainty in the data base. This also would be tempered by the existing data base. Section 4.3. discusses the data base from the perspective of confidence in the RfD.

The information available in an incomplete data base also may indicate that the RfD should be provisional pending further investigations. For example, the U.S. Food and Drug Administration (1982) suggests that if a chemical tested in a subchronic study is found to cause focal hyperplasia, metaplasia, proliferative lesions or necrosis, then a carcinogenicity study in two rodent species is warranted. Likewise, if reproductive effects are found, then teratology testing also should be conducted.

3.3.2 Historical Use and Limitations of Occupational Exposure Limit Values

OEL values, particularly the Threshold Limit Value (TLV) recommended by the American Conference of Governmental and Industrial Hygienists (ACGIH), have had widespread use in risk assessment/management programs because of a lack of uniform benchmark values relevant to ambient air exposures. The use and limitations of OELs have been discussed in an issue paper, prepared by the Inhalation Technical Panel of the Risk Assessment Forum, that is supplementary to this document (U.S. Environmental Protection Agency, 1989).

OELs have historically been considered as surrogates for benchmark values for ambient exposures because they comprise the largest documented summary of toxicological, epidemiological, and clinical information pertaining to human exposure to airborne contaminants. They include the Occupational Safety and Health Administration Permissible Exposure Limits (PELs) or full text standards, the National Institute of Occupational Safety and Health Recommended Standards, and the ACGIH TLVs. OELs differ among themselves in regard to the philosophy of the sponsoring organization, legal mandate, objectives, assumptions, and evaluation of scientific data. They share the common elements of inhalation exposure and goal of protection of human health.

Although OELs represent a large body of readily available information (e.g., there are >600 OELs), there are several factors which limit their usefulness in the derivation of RfDs. First, OELs may not be established based on chronic effects and may differ from RfDs in severity of effect. Second, OELs assume intermittent exposure periods, whereas RfDs are set to protect against continuous exposure. Third, OELs may not incorporate the most current toxicological information because toxicological review is not on a regular basis. Fourth, the unavailability of unpublished corporate documentation precludes scientific scrutiny of the primary basis for a number of TLVs (Castleman and Ziem, 1988). Fifth, the evaluation of toxicity data by agencies deriving OELs may differ from that of EPA with respect to weight-of-evidence classification, application of uncertainty factors, and other issues. Finally, the use of OELs is established to protect the average healthy worker (ages 18 to 65 years) against the adverse effects of inhaled pollutants; inhalation RfDs, on the other hand, are relevant to those of any age and/or health status.

The Agency does not endorse the general use of OELs in deriving RfDs. The OEL data base should be evaluated on a case-by-case basis according to the methodology for inhalation RfD derivation. The biological endpoint, quality and nature of the underlying data sets, the exposure scenarios, and applicability to highly-sensitive subpopulations are among those factors that must be considered for relevance to nonoccupational exposures.

4. QUANTITATIVE METHODOLOGICAL PROCEDURES

4.1 PROCEDURES ADDRESSING LIFETIME EXPOSURE*

An inhalation RfD (RfD_i) has a numerical value, and hence, a quantitative nature. As will be discussed, numerous theories, assumptions, and empirical data provide the quantitative framework for the RfD_i calculations. At present, the methodology is more advanced for addressing lifetime exposure (Section 4.1), but approaches for estimating partial lifetime exposures (Section 4.2) are under development. To account for inherent uncertainties in the chemical-specific data base and essential qualitative judgements, levels of confidence (Section 4.3) are assigned, enhancing the interpretation of a numerical RfD_i.

4.1.1 Approach for RfD Estimation

RfDs are typically calculated using a single exposure level and uncertainty factors that account for specific deficiencies in the toxicity data base. Both the exposure level and the uncertainty factors are selected and evaluated in the context of all available chemical-specific literature. After all toxicological, epidemiologic, and supporting data have been reviewed and evaluated, a key study is selected that reflects optimal data on the critical effect. Dose-response data points for all reported effects are examined as a component of this review. Issues of particular significance in this endeavor include:

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*Parts of this text are excerpted from U.S. Environmental Protection Agency (1987a).

TABLE 4-1. FOUR TYPES OF RESPONSE LEVELS (RANKED IN ORDER OF INCREASING SEVERITY OF TOXIC EFFECT) CONSIDERED IN DERIVING RfD$_i$s FOR SYSTEMIC TOXICANTS

NOEL:	No-Observed-Effect-Level. That exposure level at which there are no statistically or biologically significant increases in frequency or severity of effects between the exposed population and its appropriate control.
NOAEL:	No-Observed-Adverse-Effect-Level. That exposure level at which there are no statistically or biologically significant increases in frequency or severity of adverse effects[a] between the exposed population and its appropriate control. Effects are produced at this level, but they are not considered to be adverse.
LOAEL:	Lowest-Observed-Adverse-Effect-Level. The lowest exposure level in a study or group of studies that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.
FEL:	Frank Effect Level[b]. That exposure level which produces frankly apparent and unmistakable adverse effects, such as irreversible functional impairment or mortality, at a statistically or biologically significant increase in frequency or severity between an exposed population and its appropriate control.

[a] Adverse effects are defined as any effects resulting in functional impairment and/or pathological lesions that may affect the performance of the whole organism, or that reduce an organism's ability to respond to an additional challenge.

[b] Frank effects are defined as overt or gross adverse effects (e.g., severe convulsions, lethality, etc.).

- A delineation of all toxic effects and associated exposure levels.
- Determination, to the extent possible, of effect-specific experimental threshold regions (i.e.,the NOAEL-LOAEL interface or bracket) (see Tables 4-1 and 4-2).
- Determination of the critical effect. Of the multiple toxic endpoints potentially observed, the critical effect selected is defined as the one associated with the lowest NOAEL-LOAEL bracket.
- Special consideration of species, portal-of-entry effects, and/or route-specific differences in pharmacokinetic parameters and the slope of the dose-response curve.

TABLE 4-2. RESPONSE LEVELS CONSIDERED IN DERIVING INHALATION RfDs IN RELATIONSHIP TO EMPIRICAL SEVERITY RATING VALUES. (RANKS ARE FROM LOWEST TO HIGHEST SEVERITY.)*

Effect or No-Effect Level	Rank	General Effect
NOEL	0	No observed effects.
NOAEL	1	Enzyme induction or other biochemical change, consistent with possible mechanism of action, with no pathologic changes and no change in organ weights.
NOAEL	2	Enzyme induction and subcellular proliferation or other changes in organelles, consistent with possible mechanism of action, but no other apparent effects.
NOAEL	3	Hyperplasia, hypertrophy or atrophy, but no change in organ weights.
NOAEL/LOAEL	4	Hyperplasia, hypertrophy or atrophy, with changes in organ weights.
LOAEL	5	Reversible cellular changes including cloudy swelling, hydropic change, or fatty changes.
(LO)AEL**	6	Degenerative or necrotic tissue changes with no apparent decrement in organ function.
(LO)AEL/FEL	7	Reversible slight changes in organ function.
FEL	8	Pathological changes with definite organ dysfunction that are unlikely to be fully reversible.
FEL	9	Pronounced pathologic changes with severe organ dysfunction with long-term sequelae.
FEL	10	Death or pronounced life shortening.

*Adapted from DeRosa et al. (1985) and Hartung (1986).

**The parentheses around the "LO" in the acronym "LOAEL" refer to the fact that any study may have a series of doses that evoke toxic effects of rank 5 through 7. All such doses are referred to as adverse effect levels (AELS). The lowest AEL is the (LO)AEL.

The threshold concept is the basis for the derivation of the RfD. Essentially, an experimental exposure level is selected from the available studies which represents the highest level tested in which the critical effect was not demonstrated. Conversion of experimental exposure levels to human equivalent concentration ($NOAEL_{[HEC]}$) estimates, by adjustment for dosimetric differences between the experimental species and humans, should be made before these choices are performed (see Section 4.1.1.2 and Appendices G, H, I). This chosen human equivalent concentration ($NOAEL_{[HEC]}$) represents the first quantitative basis for the scientific evaluation of the risk posed to humans by noncancer toxicants. The inhalation RfD is operationally derived from this $NOAEL_{HEC}$ by consistent application of generally order of magnitude uncertainty factors (UFs) that reflect the second quantitative basis of this scientific evaluation of risk. Uncertainty factors are associated with various specific recognized uncertainties in extrapolating from the type of study serving as the basis for the RfD to the scenario of interest for the risk assessment. An additional modifying factor (MF) reflects professional judgment of the entire data available on the specific agent (see Table 4-3).

The RfD_i is derived from the NOAEL as:

$$RfD_i = NOAEL_{[HEC]}/(UF \times MF) \tag{4-1}$$

where:

$NOAEL_{[HEC]}$ = NOAEL, adjusted for dosimetric differences between animal species and humans, expressed as human equivalent concentration,

UF = an uncertainty factor suited to the characteristics of the data (Table 4-3), and

MF = a modifying factor based on professional judgment of the entire data base (e.g., sample size).

In general, the choice of these factors reflects the uncertainty associated with estimation of an RfD from different human or animal toxicity data bases. For example, if sufficient data from chronic duration exposure studies are available on the threshold region of a chemical's critical toxic effect in a known sensitive human population, then the UF used to estimate the RfD may be 1. That is, these data are judged to be sufficiently predictive of a population subthreshold dose, so that additional UFs are not needed.

TABLE 4-3. GUIDELINES FOR THE USE OF UNCERTAINTY FACTORS IN DERIVING REFERENCE DOSE (RfD)*

Standard Uncertainty Factors (UFs)		
H	Human to sensitive human	Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population.
A	Animal to human	Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of average healthy humans.
S	Subchronic to chronic	Use up to an additional 10-fold factor when extrapolating from less than chronic results on experimental animals or humans when there are no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs.
L	LOAEL to NOAEL (refer also to Table 4-1)	Use up to an additional 10-fold factor when deriving an RfD from a LOAEL, instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELS to NOAELs.
**D	Incomplete to complete data base	Use up to a 10-fold factor when extrapolating from valid results in experimental animals when the data are "incomplete." This factor is intended to account for the inability of any single animal study to adequately address all possible adverse outcomes in humans.
Modifying Factor (MF)		
		Use professional judgment to determine another uncertainty factor (MF) that is <10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above; e.g., the number of animals tested. The default value for the MF is 1.

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*Adapted from: U.S. Environmental Protection Agency, 1987a.

**Use of this UF is now undergoing discussion in Risk Assessment Forum (see also discussion in Section 4-3).

A UF of 10 is generally used to estimated RfDs with appropriate chronic human data, and reflects intraspecies human variability to the adverse effects of a chemical (i.e., H in Table 4-3). A UF of 100 is generally used to estimate RfDs with chronic animal data, thereby accounting for both interhuman and interspecies variability (i.e., H x A). It is generally acknowledged that these estimates are uncertain. If specific information exists to indicate a different but more exact interhuman or interspecies extrapolation procedure for that chemical, it should be used and the rationale underlying its use clearly explained.

An RfD based on a NOAEL with satisfactory subchronic animal data would require a factor to address the uncertainty in extrapolating data from subchronic to chronic exposures (i.e., S), as well as the two former uncertainty factors (i.e., H x A).

A UF of 10 generally is applied to estimated RfDs using LOAELs if NOAELs are unavailable (i.e., L). This UF is employed to define an exposure level below the LOAEL expected to be in the range of a NOAEL.

Under some circumstances, the U.S. Environmental Protection Agency applies a UF up to 10 when the data base is deficient in some major aspect; for example, if it lacks a two-generation reproductive study (i.e., D). The U.S. Food and Drug Administration has addressed this issue with the use of a twofold safety factor. Thus, in situations where a subchronic animal bioassay was available, but information in a second experimental species was lacking, a 2,000-fold safety factor (i.e., $2_D \times 10_H \times 10_A \times 10_S$) was used to estimate an acceptable daily intake (Shibko, 1981).

It is important to note that when sufficient human data are available on a chemical's critical effect and pharmacokinetics, the UFs may be smaller than those described in Table 4-3, or unnecessary. Likewise, in cases where data do not completely fulfill the conditions for a category or UF, or appear to be intermediate between two categories, an intermediate UF is suggested to estimate the RfD (Federal Register, 1980).* When a single subchronic study that does not define a NOAEL is the only available information, the U.S. EPA recognizes that all five areas of uncertainty are present. In this case, the overall UF used is generally 10,000. This coalescing of several areas of uncertainty is based on the knowledge that each individual factor is generally conservative from the standpoint of the behavior of the average chemical (Dourson and Stara, 1983), and that the multiplication of four or five values of 10 is likely to yield unrealistically conservative RfDs.

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*Other authors have discussed these areas of uncertainty or UFs in general. The interested reader is referred to Zielhuis and van der Kreek (1979) for a discussion of these factors in setting health-based permissible levels for occupational exposure, and Dourson and Stara (1983) for a summary of these factors regarding oral exposures. Other publications include Gaylor (1983), who discusses the use of safety factors for controlling risk; Crump (1984), who discusses problems with the current methods that includes UFs; Krewski et al. (1984), who contrast safety factors and mathematical models as methods for determining "safe" levels of exposure; Calabrese (1985), who discusses UFs and interindividual variation; and Lu (1983, 1985b), who discusses safety factors from the perspective of the World Health Organization.

The areas of scientific uncertainty discussed in the preceding section do not represent all the uncertainties in a dose-response assessment; for example, the number of animals that determines the NOAEL is not normally considered in the previous factors. The fewer the number of animals used at a dose, the more likely the dose is to be a NOAEL (other factors being equivalent). The effect of small sample size has long been recognized in toxicology (Bliss, 1938) and recent research has focused on adjusting for this by taking the power of individual studies into account (Brown and Erdreich, 1989). Although never explicitly stated, when faced with such an uncertainty scientists have modified the usual 10-fold factors either up or down. For example, a 100-fold UF may be raised to 125 if the number of animals in a chronic study was fewer than thought reasonable by the risk assessor. While this evaluation is scientifically in the correct direction, it introduces two difficulties in the resulting assessment. The first is that the adjustment of the standard 10-fold values is perceived as arbitrary, and the second is that the precision of some of the resulting UFs is not at all appropriate in relationship to the underlying biology (in this example a UF of 125 has a precision of three digits).

The U.S. EPA's use of the MF is an attempt to separate the "traditional" areas of scientific uncertainty that have been quantified to some extent, from these latter areas of scientific uncertainty that have not been quantified. The intent is to arrive at the best choice of an RfD, which in many cases will include an analysis of the same overall uncertainties as addressed historically, while avoiding the perception of arbitrariness and, moreover, be consistent with the overall precision of the value.

There are certain circumstances specific to inhalation that may require changes in UFs. For example, the UF used when extrapolating from a subchronic to a chronic study is assumed to be adequate for oral studies in the great majority of cases. A UF of extrapolation of subchronic to chronic exposures for inhalation studies also should be adequate with certain exceptions.

Possible exceptions include the following:

- Exposure to chemicals that are considered likely to induce hypersensitivity (e.g., beryllium)
- Exposure to chemicals that are considered likely to induce very slowly developing ("smoldering") effects
- Exposure to inhaled relatively insoluble particulate matter where the clearance rate may slow or stop when a threshold for clearance is reached. Thus, after long-term exposure lung loads can reach much higher levels than could reasonably be expected from lower level, chronic exposure conditions

The appropriate UF for these situations should be decided on a case-by-case basis until more definitive guidelines are available.

If multiple NOAELs are available in one animal species, the highest NOAEL for that individual species is used in comparison to other species NOAELs. If multiple NOAELs for the critical effect are available in different species, the lowest of these NOAELs generally is selected as the exposure level that most closely defines the threshold for adverse effects of the dose-response curve. It is consistent with U.S. EPA policy to use data on the most sensitive animal species as a surrogate to humans unless data exists to the contrary. Often an appropriate NOAEL will not be available. In that event, other estimates of effect-specific thresholds may be used. Based on the dose-response classification system presented in Table 4-1, the following guidelines may be employed (adapted from Federal Register, EPA, 1980):

- An FEL from a study with no other dose-response levels is unsuitable for the derivation of an RfD.
- A NOEL from a study with no other dose-response levels is unsuitable for the derivation of an RfD. If multiple NOELs are available without additional data, NOAELs, or LOAELs, the highest NOEL should be used.
- A NOAEL or LOAEL may be suitable for RfD derivation. A well-defined NOAEL from at least a 90-day study may be used directly, applying the appropriate UF. In the case of a LOAEL, an additional UF (10_L) is applied.

Note: caution must be exercised not to substitute FELs for LOAELs.

- If, for reasonably closely spaced doses, only a NOEL and a LOAEL of equal quality are available, then the appropriate uncertainty factor is applied to the NOEL.

Please refer to Section 3.2 and Appendix G for a complete discussion of these issues.

4.1.1.1 Minimum Criteria. Data bases vary considerably in their completeness. With a more complete data base, the magnitude of the required UF is reduced. Well-defined and conducted subchronic toxicity studies are generally considered to be reliable predictors of many forms of toxicity with the notable exceptions of carcinogenic, teratogenic, or reproductive effects. Consequently, the minimum data base acceptable for development of an RfD is a subchronic toxicity study that clearly identifies the "threshold region" of the dose-response curve. Section 4.3 also discusses this minimum data base from the viewpoint of distinguishing between high and low confidence in the RfD.

It should be recognized, however, that for some substances, results of other studies may suggest the possibility of effects not detected in the subchronic studies that constitute this minimum data base. When such findings are reported, it is desirable to consider the results of the risk assessment as tentative, indicate that the confidence in the estimate is low, and pursue additional toxicity testing. For example, if a compound tested in a subchronic study is found to cause focal hyperplasia, metaplasia, proliferative lesions, or necrosis, then a cancer bioassay is clearly indicated. Alternatively, if a subchronic study demonstrates reproductive organ toxicity or neurotoxic effects, reproductive/teratologic or neuropathology studies may be appropriate.

Extrapolation from subchronic to chronic exposure conditions (S in Table 4-3) necessitates the utilization of an additional UF of 10 in most cases. Empirical evidence supports the proposition that subchronic toxicity data can be used in this way for risk assessment purposes. McNamara (1976) has demonstrated that a 10-fold factor applied to a subchronic NOEL would predict a chronic NOEL for 95 percent of the 122 compounds for which both chronic and subchronic data for the oral route of exposure were available. To the degree that route-specific and duration-specific data are not available, increased reliance on additional extrapolation assumptions and larger UF is necessary.

In summary, with more extensive data the threshold region of the dose-response curve is more reliably approximated and the magnitude of the associated uncertainty in the risk assessment is reduced. For this reason it is desirable to state qualitatively the confidence level attached to the RfD, and the study from which the NOAEL was selected, and to rate the overall data base as high, medium, or low, as described in Section 4.3.

4.1.1.2 Calculation of Human Equivalent Concentrations . Extrapolation of animal inhalation data to humans requires estimation of the "dose" (i.e., agent mass deposited per unit tissue volume considered along with physiological and biological factors) delivered to specific target sites in the respiratory tract or made available to uptake and metabolic processes for systemic distribution (Martonen and Miller, 1986). To this end, physiologically based pharmacokinetic (PB-PK) and mathematical dosimetry models have evolved into particularly useful tools for predicting disposition differences for risk assessment (Miller et al., 1987b). Their use is predicated on the assumption that an effective (target-tissue) dose in a particular species is expected to be equally effective when achieved in some other species. However, it is likely that species differences in sensitivity occur due to such species-specific factors as host defense, repair processes, and genetics, so that the use of a ten-fold UF to account for interspecies variability, despite application of dosimetric adjustments, requires additional research. This section outlines the methods for calculating HECs estimates by using adjustment factors that have resulted from similar modeling efforts of species dosimetric differences. The factors are used to adjust the observed exposure effect levels (i.e., NOAELs, LOAELs, etc.) in animals to estimate a concentration that would be an equivalent exposure to humans. These human equivalent concentrations then can be the basis for comparison and choice of the critical effect and study as discussed in Appendix G.

Figure 4-1 is a flowchart for the calculation of HECs and provides an outline for the contents of this section. Conversion of units from ppm to mg/m^3 is required before dosimetric adjustments can be applied and this calculation is discussed in Section 4.1.1.2.1. The next step in calculating a HEC is to convert the exposure regimen of the experiment in question to that of the human exposure scenario; that is, a continuous (24-hour) lifetime (70-year) exposure, as described in Section 4.1.1.2.2. The third phase of the approach is to apply the dosimetric adjustments appropriate for the type of agent to be assessed (particle or gas/vapor), and the effect to be assessed (respiratory tract or extrarespiratory toxicity beyond the respiratory tract [systemic] resulting from an inhalation exposure). The dosimetric adjustments to derive HECs for respiratory tract effects and extrarespiratory effects of particles are provided in Sections 4.1.1.2.3.1 and 4.1.1.2.3.2, respectively. The dosimetric adjustments to derive HECs for respiratory tract effects of gases

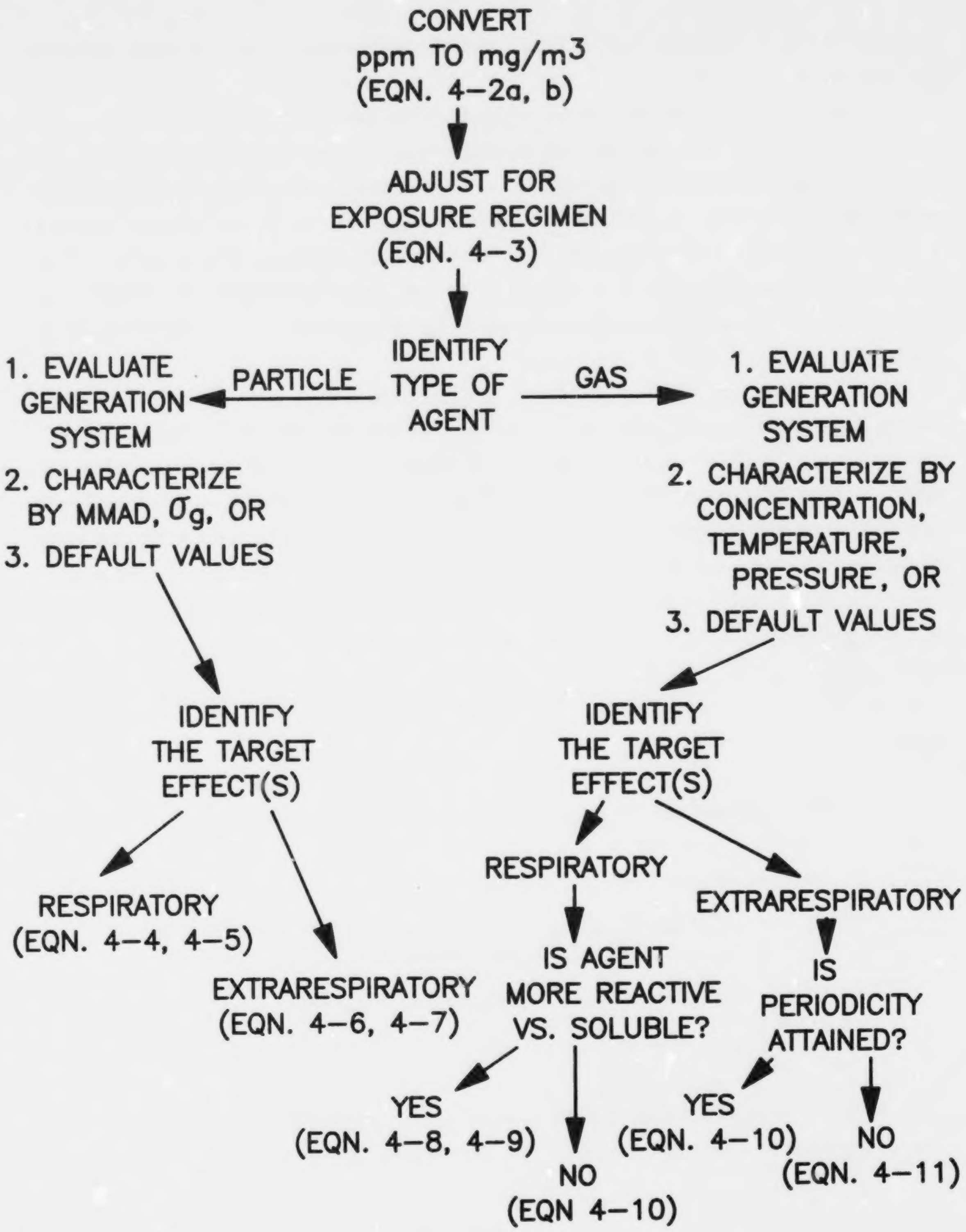
CONVERT
ppm TO mg/m3
(EQN. 4-2a, b)
ADJUST FOR
EXPOSURE REGIMEN
(EQN. 4-3)
IDENTIFY
TYPE OF
AGENT
PARTICLE
GAS
1. EVALUATE GENERATION SYSTEM
2. CHARACTERIZE BY MMAD, σg, OR
3. DEFAULT VALUES
1. EVALUATE GENERATION SYSTEM
2. CHARACTERIZE BY CONCENTRATION, TEMPERATURE, PRESSURE, OR
3. DEFAULT VALUES
IDENTIFY THE TARGET EFFECT(S)
RESPIRATORY
(EQN. 4-4, 4-5)
EXTRARESPIRATORY
(EQN. 4-6, 4-7)
IDENTIFY THE TARGET EFFECT(S)
RESPIRATORY
EXTRARESPIRATORY
IS AGENT MORE REACTIVE VS. SOLUBLE?
YES
(EQN. 4-8, 4-9)
NO
(EQN 4-10)
IS PERIODICITY ATTAINED?
YES
(EQN. 4-10)
NO
(EQN. 4-11)

Figure 4-1. Flowchart for calculation of Human Equivalent Concentrations.

are discussed in Section 4.1.1.2.4.1 and for extrarespiratory effects of gases in Section 4.1.1.2.4.2.

Although the presentation in this section divides the dosimetry calculations into those applied to extrapolate respiratory tract effects vs. extrarespiratory effects, it should be recognized that there is no strict compartmentalization of effects of a given chemical. A given inhaled chemical could cause both respiratory tract effects and extrarespiratory effects. Thus, the decision on which of the equations to use in this chapter is governed by the endpoint of interest in concert with the properties of the chemical to be assessed.

4.1.1.2.1 Dose conversion: Units. In the rare event that investigations using particulate exposures would report the concentration in ppm, a mass-density relationship would be used to convert the exposure concentration to mg/m^3. Inhalation toxicity studies on gases typically employ exposure levels expressed as mg/m^3 and/or ppm. Exposure levels for gases, including the NOAEL selected for RfD_i derivation, should be expressed in standard units of mg/m^3. For exposure levels expressed as ppm, the Ideal Gas Law can be used to derive the corresponding mg/m^3 level:

$$\frac{mg}{m^3} = ppm \times \frac{g\text{-mole}}{22.4\ \ell} \times \frac{MW}{g\text{-mole}} \times \frac{273^\circ}{T} \times \frac{P}{760\ mm\ Hg} \times \frac{10^3 \ell}{m^3} \times \frac{10^3 mg}{g} \qquad (4\text{-}2a)$$

where:

ppm = concentration expressed on a volumetric basis $\frac{\ell}{10^6 \ell}$

MW = molecular weight in grams,

22.4 ℓ = the volume occupied by 1 g-mol of any compound in the gaseous state at 0°C and 760 mm Hg,

T = actual temperature in degrees Kelvin, and

P = actual pressure in mm Hg.

At 25°C and 760 mm Hg, 1 g-mole of a perfect gas or vapor occupies 24.45ℓ. Therefore, under these conditions, the conversion becomes:

$$mg/m^3 = \frac{ppm \times MW}{24.45} \qquad (4\text{-}2b)$$

4.1.1.2.2 Dose adjustments for discontinuous exposure protocols. Many inhalation toxicity studies entail exposure regimens that are discontinuous. Often exposures are for 6-8 hours/day and 5 days/week. RfD_is are constructed to reflect a benchmark level for continuous exposure. By extension, the RfD_i also is assumed to be protective for discontinuous exposures at the same air concentration. A normalization to some given exposure (e.g., 24 hours/day for a lifetime of 70 years) is needed to adjust for the wide variety of experimental exposures to permit comparisons between studies. As discussed earlier, the RfD_i proposed herein is to reflect lifetime continuous exposure, and this scenario is the objective of normalization. Attention should be paid to the degree the applied situation deviates from the experimental, and to the physicochemical (solubility and reactivity) parameters of the inhaled agent and species-dependent factors (e.g., distribution volumes and metabolic pathways) that might temper this conversion. To calculate duration-adjusted exposure levels in mg/m^3 for experimental animals, the appropriate equation is:

$$NOAEL_{[ADJ]}(mg/m^3) = E(mg/m^3) \times D(hours/day/24\ hours) \times W(days/7days) \qquad (4\text{-}3)$$

where:

E = experimental exposure level,
D = number of (hours exposed/day)/24 hours, and
W = number of (days of exposure/week)/7 days.

Use of extreme caution is emphasized with this conversion equation, especially as the effect in question increases in its severity. The toxicity of an exposure is fundamentally dependent upon the character of the "concentration-time" (C x T) curve, which is a hyperbola whose arms converge asymptotically toward the axes of the coordinates (Bliss, 1940). Bliss and James (1966) have shown that such curves can be extrapolated with minimal error when the time points in the experiment are located on the segment of the curve asymptotically approaching the axes of the coordinates. The exposure duration should ideally embrace the time span in which the rate of onset of specific toxic effects sharply change, reflecting the degree of arc in the curve of the (C x T) relationship. Fiserova-Bergovera et al. (1980), using a compartmentalized model based on first-order kinetics, demonstrated that duration of exposure to a gas can have profound effects on the fractions of uptake exhaled or metabolized. Concentrations in tissues reflected the concentration variations in exposure, but the variation in tissues was greater during exposure to low solubility gases than to lipid soluble vapors (blood to air partition coefficients of 0.5 and 10.0, respectively), due to the faster equilibration of partial pressures of the low solubility gases. Variations between tissue and exposure concentrations were diminished if the substances were metabolized. Since the toxic effect is related to tissue concentration, consideration should be given to these duration and solubility effects. Extrapolation should be attempted only if a steady-state was attained. Likewise, linear extrapolation from one concentration exposure to another is possible only if all processes involved in the uptake and elimination of the inhaled agent are first order. Differences are caused primarily by concentration-dependent metabolic clearance (Fiserova-Bergerova et al., 1987). Limitations of this type of conversion also are discussed in Section 2.2.

4.1.1.2.3 Dosimetry: Particles. Inhalation toxicologists have advanced their ability to measure the deposition values for particles in the various regions of the lungs across species. Initially the data were primarily total deposition values for polydisperse and sometimes unstable aerosols, but data now exist for insoluble monodisperse aerosols of different sizes under different breathing conditions (U.S. Environmental Protection Agency, 1982). Data are available across most experimental species of interest on the regional deposition of applicable particle size ranges and on the necessary physiologic parameters (e.g., tidal volumes and regional surface areas) incorporated in dose adjustments (Overton et al., 1987; Miller et al., 1987b; Miller et al., 1988; Raabe et al., 1988; Patra et al., 1986; Patra, 1986). Deposition data are usually presented or modeled as the deposition fraction for each respiratory tract region of the species of interest. Deposition fraction is the ratio of the number or mass of particles deposited in the respiratory tract to the number or mass of particles inhaled, as illustrated in Figures 2-2 and H-1 [B]. Deposition data also may be normalized for the percent entering a region, particularly for the tracheobronchial region. Although not presented in the approach outlined below, iterative calculations are available to make normalized data amenable to the deposition fraction application (Miller et al., 1988). Refer to Appendix H for an explanation of these calculations.

A vast amount of knowledge also has been gained in the technology and methods for generating and characterizing aerosols. State-of-the-art inhalation toxicology studies will have characterized the particulate exposure by a given particle diameter (e.g., D_{ae}, D_{ar}, MMAD) and the geometric standard deviation (σ_g). The distribution of particle sizes for the aerosol then can be conveniently described (and/or graphically plotted as in Figures 2-5 and H-1[A]) as a probability density function.

Because of these advances in quantitation of species-specific regional respiratory tract deposition and physiologic parameters, the following describes how interspecies dosimetric comparisons can be made using data typical for particles. This application is an adaptation (Miller et al., 1983b; Graham et al., 1985) and is limited at this time to relatively insoluble and nonhygroscopic particles. The calculations to derive HECs lung effects and extrarespiratory effects of particles will then be discussed in Sections 4.1.1.2.3.1 and 4.1.1.2.3.2, respectively.

The product of deposition efficiency and particle distribution curves can be integrated to compute the deposited dose of exposure particles in a given region of the respiratory tract for the experimental species in question. That is, for each particle size range, the product of the particle distribution and deposition fraction in that range can be computed for a given respiratory tract region. Summation of these products across all the particle size ranges yields an estimate of the mass deposited in the region. These estimates then can be adjusted for ventilation parameters and lung surface area to calculate the regional deposited dose (RRD) in mg/cm^2 of respiratory tract per minute. Determining the RDD in this manner for each species allows regional deposited dose ratios (RDDR) to be calculated in order to adjust the exposure effect level for dosimetric differences between the experimental species and humans.

Notationally, for the i^{th} size range of an exposure aerosol with a given particle diameter and σ_g, let

P_i = the particulate mass fraction in that size range, and

E_i = the deposition efficiency for the species and respiratory tract region (i.e., extrathoracic, tracheobronchial and/or pulmonary, or total) of interest;

then the RDD expressed as mg/cm^2 of respiratory tract region per minute can be computed as:

$$\mathrm{RDD} = \frac{10^{-6}\ YV_Tf}{S} \sum_{i=1}^{n} P_i\ E_i \qquad (4\text{-}4)^*$$

where:

n = number of size ranges,

Y = exposure level (mg/m^3),

V_T = tidal volume (mℓ),

f = breathing frequency (breaths/minute), and

S = regional surface area (cm^2) of toxic effect observed.

This RDD can be calculated for each region of interest; that is the extra-thoracic (RDD_{ET}), the tracheobronchial (RDD_{TB}), the pulmonary (RDD_{PU}) region the thoracic (RDD_{TH}) or the total respiratory tract (RDD_{TOT}). It should be calculated according to the effect of interest. For example, the RDD summed across the TB and PU regions, the thoracic RDD (RDD_{TH}), would be used to compute the RDD for assessment of a "lung effect" ($RDD_{TH} = RDD_{TB} + RDD_{PU}$); whereas the RDD_{ET} alone would be calculated for an effect concerning the nasal turbinates.

The RDD in each species then can be used to adjust the exposure effect level for dosimetric differences between species by calculating the

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*This is an adaptation (Miller et al., 1983b and Graham et al., 1985) limited to insoluble and nonhygroscopic particles only.

RDDR, defined as the ratio of RDD in the animal species of interest (subscript A) to that of humans (subscript H) as:

$$\text{NOAEL}_{[\text{HEC}]}\ (\text{mg/m}^3) = \text{NOAEL}_{[\text{ADJ}]}\ (\text{mg/m}^3) \times \text{RDDR} \qquad (4\text{-}5)$$

where:

$\text{NOAEL}_{[\text{HEC}]}$ = the NOAEL human equivalent concentration,

$\text{NOAEL}_{[\text{ADJ}]}$ = the NOAEL adjusted for duration according to Equation 4-3, and

RDDR = $(\text{RDD})_A/(\text{RDD})_H$, the ratio of regional deposited in animal species to that of humans for region of interest for the toxic effect.

Appendix H describes the derivation of the RDD values for humans and discusses the surface area values used for both animals and humans. The surface area values used are the best available estimates for the various species at this time. Research as described in Appendix H under Research and Development may provide estimates of greater accuracy as the methodology develops. Appendix H also provides a table for the calculation of RDDR for rats and an example application of its use in dosimetric adjustment.

4.1.1.2.3.1 Respiratory tract effects. The general dosimetric approach for insoluble aerosols outlined above provides the basis for calculations for estimating HECs when the toxic effect of interest is in the respiratory tract. The equivalent dose across species is assumed to be the aerosol mass (mg) per surface area (cm^2) of the respiratory tract region of concern.

The initial step of the calculation is to characterize the particulate exposure by its MMAD and σ_g. This information will be used in conjunction with deposition efficiency to calculate a regional deposited dose. The respiratory tract region of the observed toxic effect dictates the RDD calculated. For example, if the toxic effect of interest was an effect on the nasal epithelium, Equation 4-4 would be modified to calculate the RDD for that region only as:

$$RDD_{ET} = \frac{10^{-6}\ YV_T f}{S_{ET}} \sum_{i=1}^{n} P_i\ E_i \qquad (4\text{-}4)$$

where:

P_i = the particulate mass fraction in the exposure size distribution (MMAD, σ_g)

E_i = the deposition efficiency of that size distribution (MMAD, σ_g) in the extrathoracic region for the species of interest,

n = number of size ranges,

Y = exposure level (mg/m^3),

V_T = tidal volume (mℓ),

f = breathing frequency (breaths/minute), and

S_{ET} = surface area of the extrathoracic region (cm^2).

The RDD in the species that exhibited the ET effect then is related to the human RDD, also calculated for the ET region and the same MMAD and σ_g, as a ratio. This ratio then is used as in Equation 4-5, to calculate a human equivalent concentration for the exposure NOAEL as follows:

$$NOAEL_{[HEC]}\ (mg/m^3) = NOAEL_{[ADJ]}\ (mg/m^3) \times RDDR_{(ET)} \qquad (4\text{-}5)$$

where:

$NOAEL_{[HEC]}$ = the NOAEL human equivalent concentration,

$NOAEL_{[ADJ]}$ = the NOAEL adjusted for duration according to Equation 4-3, and

RDDR = $(RDD_{ET})_A/(RDD_{ET})_H$, the ratio of regional deposited dose in the extrathoracic region in the animal species to that of humans.

4.1.1.2.3.2 Extrarespiratory effects. When the toxic effect of interest for RfD_i evaluation is observed outside the respiratory tract, the following equation is used to calculate the RDD expressed as mg/kg per minute:

$$RDD_{ER} = \frac{10^{-6}\, YV_T f}{BW} \sum_{i}^{n} P_i\, E_i \qquad (4\text{-}6)$$

where:

P_i = the particulate mass fraction in the exposure size distribution (MMAD, σ_g),

E_i = the deposition efficiency of that size distribution (MMAD, σ_g) in the entire respiratory tract for the species of interest,

n = number of size ranges,

Y = exposure level (mg/m^3),

V_T = tidal volume (mℓ),

f = breathing frequency (breaths/min), and

BW = body weight (kg).

In the case of extrarespiratory effects of particles, the equivalent dose across species is assumed to be the mass of particles (mg) deposited per body weight (kg). Until clearance and distribution parameters can be incorporated, it is assumed that 100 percent of the deposited dose to the entire respiratory system is available for uptake to the systemic circulation. This assumption may result in slightly less conservative HEC estimates than using retained dose and accounting for differential uptake from various respiratory regions, but is more accurate than using the exposure concentration.

The ratio of the extrarespiratory RDDs calculated for the experimental species and the human then is used to calculate the HEC for a systemic effect as follows:

$$\mathrm{NOAEL}_{[\mathrm{HEC}]}\ (\mathrm{mg/m^3}) = \mathrm{NOAEL}_{[\mathrm{ADJ}]}\ (\mathrm{mg/m^3}) \times \mathrm{RDDR}_{\mathrm{ER}} \qquad (4\text{-}7)$$

where:

$\mathrm{NOAEL}_{[\mathrm{HEC}]}$ = the NOAEL human equivalent concentration,

$\mathrm{NOAEL}_{(\mathrm{ADJ})}$ = the NOAEL adjusted for duration according to Equation 4-3, and

$\mathrm{RDDR}_{\mathrm{ER}}$ = $(\mathrm{RDD}_{\mathrm{ER}})_A/(\mathrm{RDD}_{\mathrm{ER}})_H$, the ratio of the dose available for uptake from the entire respiratory system of the experimental animal species to that of humans.

4.1.1.2.3.3 Assumptions and default values. The initial step in the calculation of HECs, after evaluation of the generation system for its adequacy, involves characterization of the aerosol exposure by its MMAD and σ_g. Studies that do not provide this information should be suspect for deficient quality. Some of the older toxicology literature may not provide this information, however, and a default value may need to be invoked. The first approach in this situation is to attempt an estimate of particle size and distribution based on the generation apparatus used. In conjunction with this information, the knowledge that prior to the late 1970s, the generation technology was not sufficiently sophisticated to deliver consistent exposures of particle sizes above 3 μm (MMAD) can be used to construct a default approach. The recommended default approach is to use the particle diameter (MMAD) and distribution (σ_g) characteristic for the given generation system that is $\leq$3 μm and that yields the smallest (i.e., most conservative) RDDR values for the lung region of interest. The Hatch-Choate equations can be used to convert lognormal distributions of one type of diameter (e.g., count median diameter) to another (e.g., MMAD) (Hinds, 1982).

The MMAD for liquid and hygroscopic particles may vary with location in the respiratory tract since its size, shape, and density may change due to water uptake in the humid respiratory tract. Consequently, the deposited dose may be different from that of nonhygroscopic particles of like size distribution upon inhalation (Martonen et al., 1985). Theoretical models have been developed to analyze the influences of hygroscopic growth on inhaled aerosol behavior (Martonen et al., 1985; Martonen, 1982; Martonen and Patel, 1981), but application in risk assessment awaits definition of the primary factors influencing hygroscopic growth on species- and agent-specific bases. The factors include initial particle geometry and density, material hygroscopic growth characteristics, respiratory parameters, and temperature and relative humidity profiles. Observations on the data from modeling efforts to date indicate that hygroscopic particles in the diffusion-dominated regime have reduced deposition relative to nonhygroscopic particles of identical pre-inspired size, whereas those hygroscopic particles affected by inertial and gravitational forces have an increase in deposition relative to nonhygroscopic particles (Martonen et al., 1985). These observations may be explained by changes in the relative effectiveness of the particle deposition efficiency mechanisms. Thus, dosimetric adjustment of an inhaled dose by the deposition efficiency for nonhygroscopic particles would underestimate (i.e., be more conservative than) the deposited dose for the larger (affected by inertial and gravitational forces) hygroscopic particles, and overestimate the deposited dose for the smaller diffusion-dependent hygroscopic particles. The total deposited dose of inhaled nonhygroscopic particles, however, is always less than the initial total dose (exposure dose). Also, the relative changes in deposition will be in a similar direction in experimental animal species and humans. Dosimetric adjustment by the insoluble (nonhygroscopic) deposition efficiencies is recommended as a conservative default for the hydroscopic particles, pending modification by the elucidation of the hygroscopic models.

It is recognized that this approach is based on deposition efficiency data obtained or derived under a particular set of ventilatory parameters; that is, the experimental parameters for the animal and a derived human breathing pattern (13.8 ℓ/min or 20 m^3/day). The assumption in this application is that it is valid to linearly extrapolate from these values to other sets of breathing parameters. The parameters of this assumption, such as the effect of activity pattern and allometric relationships between lung weight, lung surface area and body weight (Adolph, 1949; Weibel, 1972; U.S. Environmental Protection Agency, 1988c) will be investigated as part of this methodology development. A discussion of the impact that breathing pattern has on the human deposition estimates can be found in Appendix H. Also, the human ambient exposure scenario, when known, may be characterized by a different MMAD and σ_g than that used to derive the health risk assessment. Comparisons between ratios calculated with a MMAD and σ_g the same as the animal exposure and calculated with the human estimate using the anticipated ambient MMAD and σ_g may provide some insight on the uncertainty of this extrapolation.

In addition to inspired air concentration, minute volume respiration rate, surface area, and deposition efficiency, the effective dose of inhaled particulate matter will vary with bioavailability. The fraction of particulate matter dissolved and assumed to be bioavailable can be expected to increase with greater particle solubility, as well as with longer residence time in the lungs. The U.S. EPA has recognized the importance of incorporating clearance components to its RDDR exposure concentration adjustments, particularly for estimates of long-term lung burdens. In addition, consideration will also be given to the issues concerning bioavailability as discussed in Appendix H.

4.1.1.2.4 Dosimetry: Gases and vapors. The approach outlined in the insoluble particle application illustrates the feasibility of interspecies dosimetry calculations for extrapolating the toxicological results of inhaled agents to human exposure conditions for risk evaluation. Dosimetry data facilitates evaluation of concentration-response data with respect to dose-response relationships. Dosimetry models also should be developed to account for the physical, biological, and chemical factors that affect gas uptake and the clearance mechanisms for various inhaled agents. Predictive physiologically based modeling for reactive gases has been demonstrated (Overton and Miller, 1988). Predictive physiologically based modeling has also been demonstrated for gases and vapors of organic solvents that may be metabolically activated (Fiserova-Bergerova, 1983; Andersen et al., 1987; Overton, 1989). For these agents, the uptake and distribution of the parent compound depends on the physicochemical properties of the agent (i.e., solubility in blood and tissue) and physiological properties (i.e., ventilation, perfusion, tissue mass). The toxicological effects can be a function of the parent compound or are a function of metabolism of the parent compound to a toxic metabolite, which depends on the rate of toxification and detoxification reactions. Consideration should be given to the discussion by the National Research Council (1986) on interspecies extrapolation based on mechanism of action. Three classes were distinguished based on whether the parent compound, stable metabolite, or reactive metabolite produces the toxic effect and suggests measures of dose for each of these classes. These factors are often species-specific and dose-dependent, as well as being chemical-specific and, therefore, require a substantial data base (beyond that which exists in most circumstances) in order to model comparative species dosimetry of gases based on mechanism of action. A project is underway by ECAO-RTP and HERL to identify the key determinants of uptake and tissue dose for a variety of gases with different properties (see "Research and Development", Appendix I). Identification of the limiting anatomic and physiologic parameters, physicochemical characteristics, and exposure concentration and duration conditions will facilitate the application of these models routinely to interspecies dose adjustments.

4.1.1.2.4.1 Respiratory tract effects. For gases and vapors that are very reactive and that have their toxic effect in the lung, an analogous approach to that of the insoluble particles approach for repiratory tract effects is used. The equivalent dose across species again is assumed to be the mass (mg) of toxic agent per surface area (cm^2) of the lung region of concern. Ventilatory parameters and regional lung surface areas are used to dosimetrically adjust for the species differences, as in Equations 4-4, but the particle distribution and deposition efficiency integration term is dropped. Thus, the regional gas dose, (RGD), is calculated as:

$$RGD = \frac{10^{-6}\, YV_T f}{S} \qquad (4\text{-}8)$$

where:

Y = exposure level (mg/m^3),

V_t = tidal volume (mℓ),

f = breathing frequency (breaths/minute), and

S = regional surface area (cm^2) of toxic effect observed.

It should be noted that this approach assumes that the entire inspired concentration goes to the region of concern, whereas not all inspired gas is necessarily deposited. For example, an alveolar ventilation rate would be appropriate to use with a strictly pulmonary effect. As in the case of the RDD for aerosols, the toxic effect observed will dictate the RGD to calculate. That is, the appropriate surface area (i.e., ET, TB, PU, TH, or TOT) must be used in Equation 4-8 to correspond with the region of observed toxicity. The ratio of the appropriate RGDs, calculated for the experimental species and humans, is then derived. This regional gas dose ratio (RGDR) then is used to dosimetrically adjust the experimental NOAEL to a human equivalent concentration:

$$\text{NOAEL (mg/m}^3)_{[\text{HEC}]} = \text{NOAEL}_{[\text{ADJ}]}\ (\text{mg/m}^3) \times \text{RGDR} \qquad (4\text{-}9)$$

where:

$\text{NOAEL}_{[\text{HEC}]}$ = the NOAEL HEC

$\text{NOAEL}_{(\text{ADJ})}$ = the NOAEL adjusted for duration according to Equation 4-3, and

RGDR = $(\text{RGD})_A/(\text{RGD})_H$, the ratio of regional gas dose in animal species to that of humans for region of interest for the toxic effect.

For gases with respiratory tract effects that have significant solubility in the blood relative to their reactivity with lung tissue (e.g., methyl bromide), the approach outlined below for gases which reach periodic concentrations and cause extrarespiratory effects is recommended (Equation 4-10). This default is used to account for uptake into the systemic circulation which may have decreased the amount of gas causing a direct effect in the lung and to account for the concentration available to the lung via blood circulation.

4.1.1.2.4.2 Extrarespiratory effects. For gases and vapors that exhibit their toxic effects outside of the respiratory tract, an approach for the scenario when the arterial concentration (leaving the lung) of the gas in the animal was periodic (or could be expected to be) with respect to time (Equation 4-10) is recommended. A default approach for the case when such periodicity is suspected not to have occurred also is provided (Equation 4-11).

Derivation of the procedure and Equation 4-10 for estimating $\text{NOAEL}_{[\text{HEC}]}$s for gases with extrarespiratory effects was based on a PB-PK model described in Appendix I. The procedure will give equivalent or more conservative values for the $\text{NOAEL}_{[\text{HEC}]}$s than those obtained by using the PB-PK model, and can be used with compounds for which modeling would be applicable, but for which some or all values of the important parameters (λ, V_{max}, K_m) are not available. The approach assumes that physiologic and kinetic processes can be described by a PB-PK model, assumes allometric scaling of physiologic and kinetic parameters, and assumes that all concentrations of the inhaled compound within the animal are periodic with respect to time. Based on the PB-PK model of Ramsey and Andersen (1984), algebraic equations that relate organ and tissue compartment concentrations to exposure concentrations under equilibrium conditions were derived. Since toxic effects observed in chronic bioassays are the basis for the determination of NOAELs from which RfD values for human exposures are derived, the procedure assumes that chronic animal exposure scenarios are equivalent to human lifetime exposures. The procedure also assumes that the toxic effects observed are related to the arterial blood concentration of the inhaled compound and that $\text{NOAEL}_{[\text{HEC}]}$s should be such that the human time-integrated arterial blood concentration is less than or equal to that of the exposed laboratory animal. This latter assumption is equivalent to assuming that time-average concentrations are equal to the equilibrium concentration adjusted for exposure duration (i.e., Equation 4-3). A mathematical derivation was used to obtain the proposed method of simple algebraic equations to compute $\text{NOAEL}_{[\text{HEC}]}$s. A more detailed description of the development of the procedure is given in Appendix I.

Assuming the animal alveolar blood concentrations were periodic with respect to time for the majority of the experiment duration, the $\text{NOAEL}_{[\text{HEC}]}$ for extrarespiratory effects of gases or vapors is calculated as:

$$\text{NOAEL}_{[\text{HEC}]}\ (\text{mg/m}^3) = \text{NOAEL}_{[\text{ADJ}]}\ (\text{mg/m}^3) \times \frac{\lambda_A}{\lambda_H} \qquad (4\text{-}10)$$

where:

$\text{NOAEL}_{[\text{HEC}]}$ = the NOAEL human equivalent concentration,

$\text{NOAEL}_{(\text{ADJ})}$ = the NOAEL adjusted for duration according to Equation 4-3, and

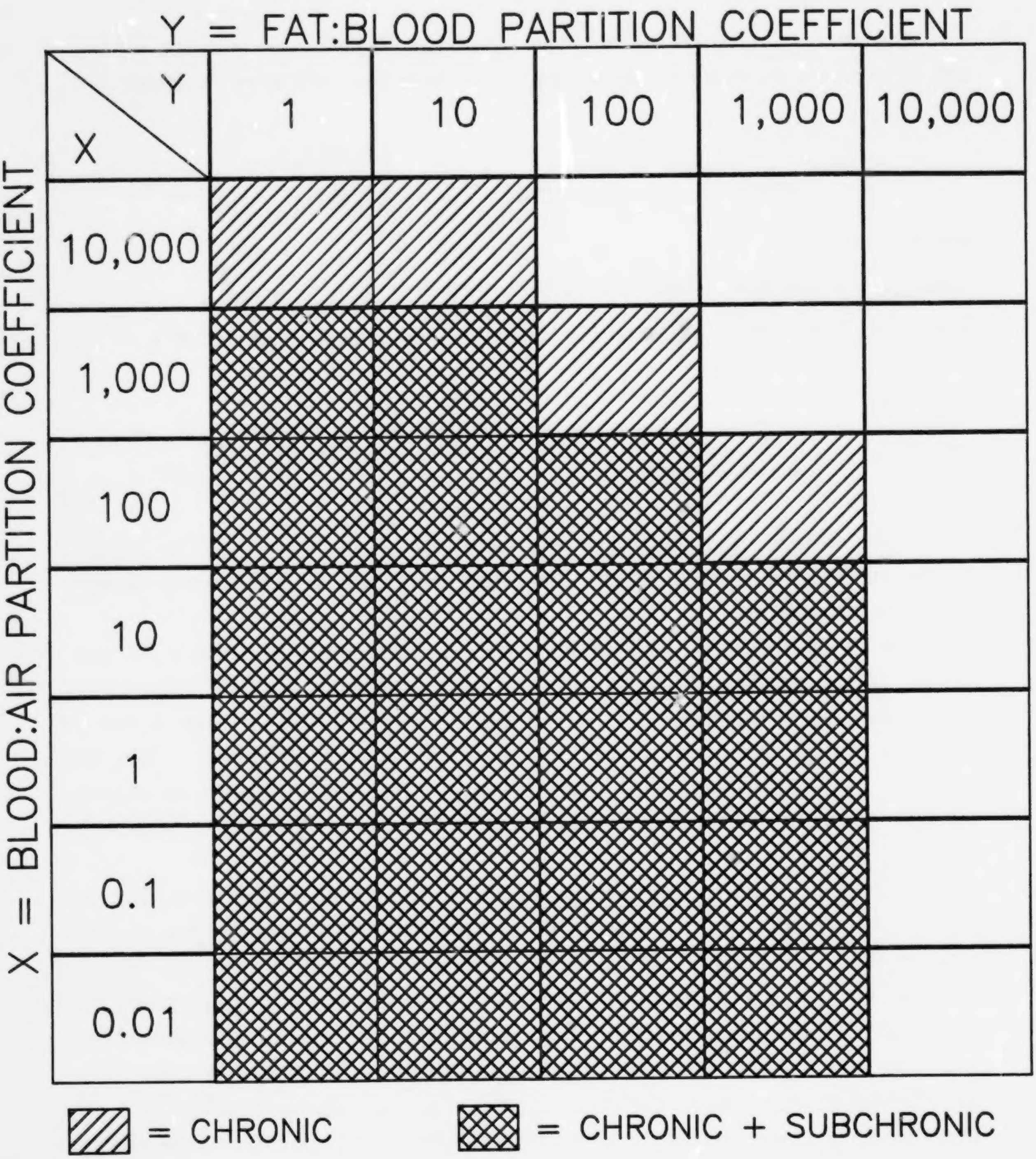
λ_A/λ_H = the ratio of the blood to air partition coefficient of the chemical for the animal species to the human value, used only if $\lambda_A \leq \lambda_H$.

For the cases where $\lambda_A > \lambda_H$, model results have shown that the generalized Equation 4-10 may not provide conservative estimates. The detailed derivation of boundary limits on λ is given in Appendix I. For the situation in which $\lambda_A > \lambda_H$ and in the case where λ values are unknown, the default value of $\lambda_A/\lambda_H = 1$ is recommended. An analysis of the available data on rats for blood to air partition coefficients shows that the λ_A is greater than λ_H in most cases. Practically, the conditions of periodicity should be met during "most" of exposure duration. For example, if this condition is met for nine tenths of the time (e.g., periodic during the last 90 weeks of a 100 week experiment), then estimates of average concentrations will be in error by less than 10%.

Figure 4-2 provides guidance on the relationship of the blood to air and fat to blood partition coefficients with respect to achieving periodicity of an inhaled agent in the arterial blood of a 380-gram F344 rat. (It should be noted that often tissue to air partition coefficients are reported, e.g., fat to air. The fat to blood partition coefficient can be calculated by multiplying the fat to air partition coefficient by the blood to air partition coefficient.) The PB-PK model as described in Appendix I was run to simulate a 6 hours/day, 5 days/week exposure regimen of 10 ppm. Physiologic parameters, such as ventilation rate, were scaled as described in Appendix I. No metabolic parameters were incorporated in the model for the simulations, since the arterial blood concentration takes longer to reach periodicity without metabolism. This figure thus represents the most conservative values for the partition coefficients for that exposure regimen. The blood to air and fat to blood partition coefficients were chosen based on sensitivity analyses that indicated these two parameters were important to describing the time course of the concentration of an agent in the arterial blood, and upon data availability.

The importance of the relationship between the partition coefficients and the attainment of periodicity is particularly significant when extrapolating from studies of different durations. For example, for an agent with a blood to air partition coefficient of 1,000 and a fat to blood partition coefficient of 100, it would be inappropriate to extrapolate from a subchronic exposure regimen since the criterion of attaining periodicity for 90% of the exposure duration is not met. Periodicity is attained with these same parameters when the study is carried out for a longer duration, however, so that the approach based on the ratio of animal to human partition coefficients can be used on a chronic study without violation of critical assumptions.

Similar matrices to Figure 4-2 can be developed for the relationship between partition coefficients and the attainment of periodicity of the agent in the arterial blood of each experimental species of interest. Use of physiologic parameters for other species and/or different exposure regimens at various concentrations will influence this relationship and should be considered when determining the extrapolation approach to use for derivation of a human equivalent concentration.



Y = FAT:BLOOD PARTITION COEFFICIENT

X = BLOOD:AIR PARTITION COEFFICIENT

Y X	1	10	100	1,000	10,000
10,000	▨	▨			
1,000	▩	▩	▨		
100	▩	▩	▩	▨	
10	▩	▩	▩	▩	
1	▩	▩	▩	▩	
0.1	▩	▩	▩	▩	
0.01	▩	▩	▩	▩	

▨ = CHRONIC

▩ = CHRONIC + SUBCHRONIC

Figure 4–2. Relationship of partition coefficients to periodicity in F344 rat arterial blood for subchronic (90–days) and chronic exposure regimens of 6 hours/day, 5 days/week.

The default calculation for the situation in which periodicity during 10% of exposure duration is suspected not to have been achieved is given by:

$$\text{NOAEL}_{[\text{HEC}]}(\text{mg/m}^3) = \text{NOAEL}_{[\text{ADJ}]}(\text{mg/m}^3) \times \frac{(\dot{V}_A/\text{BW})_A}{(\dot{V}_A/\text{BW})_H} \tag{4-11}$$

where:

$\text{NOAEL}_{[\text{HEC}]}$ = the NOAEL Human equivalent concentration,

$\text{NOAEL}_{[\text{ADJ}]}$ = the NOAEL adjusted for duration according to Equation 4-3, and

$\frac{(\dot{V}_A/\text{BW})_A}{(\dot{V}_A/\text{BW})_H}$ = the ratio of the alveolar ventilation rate (mℓ/min) divided by BW (kg) of the animal species to the same parameters for humans.

Since this default approach engenders more uncertainty and is less conservative with respect to the above approach, use of a modifying factor should be considered.

Use of the alveolar ventilation rate is recommended to account for the volume of the respiratory tract in which no gas exchange occurs; often termed the "physiologic dead space". The alveolar ventilation rate is the volume of inspired air per minute available for gas exchange with blood that enters the alveoli. Alveolar ventilation rates are approximately 67 percent of minute volumes for mice, rats, and humans (U.S. Environmental Protection Agency, 1988c).

4.1.1.2.4.3 Assumptions and default values. As with aerosols, after evaluation of the adequacy of the generation system, the initial step in the calculation of HECs is characterization of the exposure.

Gas exposures are characterized by concentration (mg/m^3), temperature, and pressure. If the concentration is expressed in ppm, the actual temperature and pressure should be used to convert the units to (mg/m^3) (see Section 4.1.1.2.1). When the actual temperature and pressure values are not provided in a study, it should be suspect for deficient quality. Some studies, however, express values already corrected for these parameters, usually corrected at 25°C and 760 mm Hg. These values are the recommended default values for temperature and pressure, respectively.

Other assumptions and default values for gas and vapor extrapolations have been discussed in Section 4.1.1.2.4.1 and 4.1.1.2.4.2 and details are provided in Appendix I.

4.1.1.3 Route-to-Route Extrapolation. Estimating equivalencies of dose-response relationships from one route of exposure to another introduces an additional uncertainty in the derivation of an inhalation RfD. Consequently, whenever possible, the inhalation RfD should be based on data involving inhalation exposures. If inhalation data are insufficient, data from other routes of exposure may be useful in the inhalation RfD derivation process, provided that portal of entry effects in the lung can be ruled out (see Section 4.3).

Oral data are the most common alternatives to inhalation data. Dose-response data from other routes of exposure, such as intravenous, intraperitoneal, subcutaneous, dermal, and intramuscular routes also may be available. Intravenous data provide reliable information on blood levels. The other routes generally have a much more limited usefulness in route-to-route extrapolation because the pharmacokinetics are, in general, poorly characterized.

When portal-of-entry effects have been ruled out, estimates of equivalent doses can be based upon the following:

- Available pharmacokinetic data for the routes of interest
- Measurements of absorption efficiency by each route of interest
- Comparative excretion data when the associated metabolic pathways are equivalent by each route of interest
- Comparative systemic toxicity data when such data indicate equivalent effects by each route of interest.

If sufficient pharmacokinetic data are available, physiologically based pharmacokinetic (PB-PK) models are particularly useful tools for predicting disposition differences due to exposure route differences. Their use is predicated on the assumption that an effective (target-tissue) dose achieved by one route in a particular species is expected to be equally effective when achieved by another exposure route or in some other species. For example, the proper measure of target-tissue dose for a chemical with pharmacologic activity would be the tissue concentration divided by some measure of the receptor-binding constant for that chemical. Such models account for fundamental physiologic and biochemical parameters such as blood flows, ventilatory parameters, metabolic capacities, and renal clearance, tailored by the physicochemical and biochemical properties of the agent in question. The behavior of a substance administered by a different exposure route can be determined by adding equations that describe the nature of the new input function. Similarly, since known physiologic parameters are used, different species (e.g., humans vs. test species) can be modeled by replacing the appropriate constants. It should be emphasized that PB-PK models must be used in conjunction with toxicity and mechanistic studies in order to relate the effective dose associated with a certain level of risk for the test species and conditions to other scenarios.

This concept can break down when considering chemicals that exhibit first-pass effects (a pharmacologic phenomenon) and/or portal-of-entry effects (a toxic response). It is imperative to rule out pulmonary portal-of-entry endpoints before attempting route-to-route extrapolation from other data. Where a chemical is known or suspected of having a first-pass effect by the tested route, or where a portal-of-entry effect is known or suspected, then route-to-route extrapolation for derivation of an RfD is not appropriate. Agents for which this approach must be used with particular caution include metals, irritants, and sensitizers. Before route-to-route extrapolations are attempted, it is strongly suggested that articles by Pepelko and Withey (1985), the National Research Council (1986), and the publication on Pharmacokinetics in Risk Assessment (National Research Council, 1987) be reviewed for a better understanding of the complexities and limitations of some of the available extrapolation methods. Limitations also are outlined in Section 4.3.

Outstanding issues in route-to-route extrapolation include the following.

- When are the available data too sparse for estimating the different route absorption parameters?
- What default positions, if any, will be used when one or both of the route-specific absorptions cannot be estimated?
- How should the different exposure regimens by the different routes (e.g., continuous vs. intermittent exposures) be dealt with?
- How should vehicle effects on the pharmacokinetics of the oral studies (e.g., ppm in diet vs. ppm in water) be dealt with?

4.1.1.4 Issues for Further Investigation. Consistent application of the procedures in this chapter will require consensus on the most appropriate data sets (e.g., species deposition data) and reconciliation of data values for use in the dosimetry calculations. Default values used among the U.S. EPA offices should be reviewed, including a discerning reevaluation of the data source, selection rationale, and application limitations. Recent documents on recommended values for use in risk assessment (U.S. Environmental Protection Agency, 1988c) and for use in physiologically based models (U.S. Environmental Protection Agency, 1988b) are useful sources of default values for parameters such as ventilation rates and body weights for use in these equations when these values are not supplied in individual investigations. Available allometric equations (Adolph, 1949; Weibel, 1972; U.S. Environmental Protection Agency, 1988b,c), relating body size to the parameters of interest such as ventilatory rates and lung surface areas also may be appropriate. Currently, a task group of the Agency's inhalation RfD verification workgroup is addressing the issue of the use of default parameters. It must be emphasized at this time that the use of default or derived values must be consistent with the dosimetric modeling parameters and approaches used in adjusting concentrations to human equivalent values, such as the parameters used to derive the regional RDDR (see discussions in Sections 4.1.1.2.3 and 4.1.1.2.4 and Appendices H and I).

4.1.2 Approach for RfD Estimation Using Human Data

4.1.2.1 Introduction. Whenever possible, a human study is selected as the critical study for derivation of an RfD to avoid the myriad problems of extrapolating from animals to humans.

When using epidemiologic data to assess risk in the context of a method designed for data on experimental animals, the dependence of epidemiologic studies on existing exposure conditions and the necessity of using noninvasive diagnostic methods present two complicating factors. One is that existing exposure levels may not include a NOAEL. Toxicologic studies are generally designed to identify the NOAEL. For ethical reasons, many clinical studies in humans often focus on exposure scenarios that are associated with minimal effects and short exposure durations, although they also may identify a NOEL. In contrast, epidemiologic studies cannot be so designed because exposure levels are dependent on existing exposures. In both controlled human and animal studies, the estimates are biased by the dose or exposure level selected or available for study. These estimates are subject to random error, the magnitude of which depends on various design aspects, such as the size of the study population or test groups, and the underlying variability of the test animals or study subjects.

The second factor to consider for epidemiological studies is that the entire spectrum of potential adverse effects cannot be evaluated, thus, it is difficult to determine the critical effect. Prospective epidemiologic studies that assess biological markers or preclinical endpoints are better sources of NOAELs to estimate the threshold region. Clinical studies may be based on low exposure levels selected by the investigator and investigate sensitive endpoints, but these studies are generally of short duration and are more useful for estimating short-term effects (see Section 4.2). The following discussion describes approaches to address these obstacles.

4.1.2.2 Selecting the Threshold Estimate. In some epidemiologic studies only severe effects such as mortality are examined. In such studies a NOAEL has inherent limitations. A study in which sensitive endpoints are evaluated may contain a LOAEL but no NOAEL. If the effect is sensitive (i.e., it occurs early in the natural history of the disease), a LOAEL may be judged suitable for use in calculating an RfD in lieu of a NOAEL, because the uncertainty of extrapolating human data for a well-defined critical effect from a LOAEL to a NOAEL is judged to be less than the uncertainty involved in extrapolating from animal data to humans. The circumstances governing this selection include deficiency in toxicologic and physiologic data bases, small sample size in the experimental studies, or physiologic or pharmacokinetic data suggesting that animal data are unlikely to be good predictors for humans. The use of the UF for extrapolating from a LOAEL to a NOAEL has been explained previously in Section 4.1.1.

The data base supporting an occupational exposure level (OEL) may be examined for data to be incorporated in the data array for analysis supporting RfD_i derivation. Caution is recommended: While the OELs are based on the concept of a biological threshold, there are no standardized criteria for the data base and safety factors used. Furthermore, the OELs are designed to protect "nearly all workers" and not the entire population. These and other limitations are discussed in the issue paper (U.S. Environmental Protection Agency, 1989).

4.1.2.3 Defining the Exposure Level. Epidemiologists cannot control the exposure levels for a study in a systematic fashion, but instead attempt to measure the levels to which the study population is exposed insofar as the measurement technology permits. In actual exposure situations, the levels vary in time and location. Epidemiologic studies can utilize a variety of parameters to characterize exposure, although in retrospective studies they are usually quite limited by the available data.

The ideal exposure measure for humans who move about in their environment is individual data, such as might be obtained with the use of a personal monitor. However, in addition to the expense and practical difficulties, this technology is available for measuring only a few chemicals. Individual exposure can be constructed by mapping the individual's time in various exposure zones, rooms, or areas. If information on levels in the environment is not available, duration of employment often is used as a surrogate for exposure.

Parameters commonly used to measure environmental levels are cumulative exposure, peak exposure level, time-weighted average, and ratio of average to peak exposure. Currently it is unclear which of these is best related to disease and under what circumstances or chemical characteristics of the agent is one parameter better than another. For example, cumulative exposure is more appropriate as half-life of a substance is increased, therefore, to derive RfD_is that identify levels of environmental exposures that are free of adverse effects, cumulative exposure or time-weighted averages are appropriate for substances with long half-lives. The circumstances can be evaluated on a case-by-case basis and different exposure parameters may be used if the rationale is presented. For conversion of units, the approach is the same as that for animal data (Equations 4-2a and 4-2b). Conversions are the same for exposure duration (Equation 4-3), again, with the same precautions as discussed. Considerations for route extrapolation would be the same as for animal data; however, it is highly unlikely that human ingestion data would be available in a form useful for quantitative risk assessment.

4.1.2.4 Uncertainty Factors for Human Data. The best data to use for calculating an RfD_i would be a population study of humans that includes sensitive individuals exposed for lifetime or chronic duration, and evaluates the critical endpoint or an appropriate early marker for the disease. A NOAEL derived from a well-done epidemiologic study of this description may require no UF. A similar study in humans that contains only a LOAEL would require the use of a factor of up to ten-fold to reduce the exposure to the range of a NOAEL (see Table 4-3, 10L). Chronic studies on populations that do not include sensitive individuals may require a 10-fold UF. For example, studies of workers are considered to contain only relatively healthy adults. A NOAEL from a study that entails subchronic exposure would require a reduction by a 10-fold UF (see Table 4-3, 10S). However, the amount of exposure in a human study that constitutes subchronic is not defined, and could depend on the nature of the effect and the likelihood of increased severity or greater percent response with duration. In the absence of data on the relationship of animal to human lifespan for predicting health effects, a linear correlation of percent lifespan is assumed. Therefore, if a chronic study in animals is 12% of lifespan, then 9 years of human exposure must be studied. Information on the natural history and progression for the diseases should be considered and explained; information on follow-up after exposure, often available in epidemiologic studies, is important.

In some cases, short-term studies of effects in humans can give important information on irritation, sensory effects, or sensitivity and reversibility, yet give no information on the effect of chronic exposure. If the data base suggests that the effective level of a short-term human study is below that which would cause chronic health effects, this can be used to derive the RfD, designated as a subchronic inhalation RfD (RfD_{si}). This is described further in Section 4.2.2.

4.2 PROCEDURES FOR ESTIMATING PARTIAL LIFETIME EXPOSURES

4.2.1 Acute

Application of the RfD_i approach to acute exposures is contingent upon determination of relevant exposure durations for humans. Documentation on this area of interest is under development in the U.S. EPA.

4.2.2 Approach for Subchronic Inhalation RfD Estimation (RfD_{si})

The RfD_{si} strictly parallels the inhalation RfD in concept. The distinction is one of exposure duration. While the RfD is specifically developed to be protective for daily exposure to a compound over the course of a lifetime, the RfD_{si} applies to specified durations that are less than lifetime. Multiple duration-specific RfDs may be developed for a compound depending upon the medium and possible exposure scenarios, as well as the needs of a particular program office. For example, the Office of Drinking Water develops oral drinking water health advisories for 1-day and for 10-day exposures.

Once the duration of a particular exposure is defined, all of the laboratory and epidemiologic data need to be evaluated in this exposure-time context. When adequate data on humans or on laboratory animals are available for the required exposure-time interval, RfD_{si} development proceeds in the same manner as described for the RfD_i (see Section 4.1). Data on humans may be available for short-term exposures even when the chronic value (RfD_i) has been based on animal data. It is important therefore to examine the available human data to ascertain whether less-than-lifetime exposures are included.

Determining exposure-time equivalencies among species is an issue requiring further investigation. Research on the boundary limits of the blood to air and blood to fat partition coefficients for establishing periodicity of arterial concentrations during intermittent exposures as described in Section 4.1.1.2.4.2, may provide some insight. These limits will be different for 90-day versus chronic bioassays. Previous discussions have utilized the concept of percent of the lifespan. For example, chronic studies often are defined as having a duration of >90 days. Whether short-term exposures should also be evaluated in terms of percent of the lifespan, physiological time, or by some other method, requires further investigation. Essentially, an index of the damage process relative to the repair process for a number of different lesion types is necessary. In addition to exposure duration, postexposure observation time is also an important issue. For example, brief exposure to certain pulmonary irritants may result in no immediately observable adverse effects, but may be linked with pulmonary pathology at a later evaluation time. No guidance is currently available concerning adequate periods of postexposure observation for acute, short-term and subchronic exposure regimens. The duration of an adequate postexposure time period may be compound-specific.

When experimental data are available only for shorter "equivalent" exposure durations than the desired duration-specific RfD_{si}, or when postexposure observation is deemed inadequate, application of a UF may be appropriate. This is similar to the application of a UF for duration when estimating a RfD_i from subchronic animal data. Criteria are needed to determine the degree of divergence between the experimental exposure duration and time to elicit effects, which would necessitate application of an additional UF. In addition, it needs to be determined if a standard factor, such as 10, would be applied whenever the criteria for duration are not met, or whether UFs of graded magnitude might be employed, depending upon the degree of divergence between the experimental exposure duration and the duration interval modeled by the RfD_{si}.

It is important to evaluate any proposed RfD_{si} in the context of all available toxicity data. Although free-standing NOELs/NOAELs* are not recommended for either RfD_i or RfD_{si} estimation, on occasion they represent the only data available. Use of a dose level well below an actual threshold value can result in an anomalous RfD_{si}, when compared to longer exposure-duration RfD_{si} or RfD_is that are based on a more complete data set. In other words, it would be inappropriate to estimate a RfD_{si} that is of smaller magnitude than an RfD_i for the same compound.

The RfD_{si} can be calculated for any required exposure interval when adequate toxicological data are available, utilizing the approaches described in Section 4.1 as shown below:

$$RfD_{si} = NOAEL_{[HEC]}/(UF \times MF) \qquad (4\text{-}12)$$

The UFs are the same as described in Section 4.1.1. except that the NOAEL from Table 4-3 would be more generally interpreted to reflect discrepancies between the available duration-specific data and the duration of the proposed RfD_{si}. This may necessitate correction for added uncertainty.

For human data, the exposure concentration associated with a human NOAEL may be utilized directly to develop a subchronic RfD_{si} in units of air concentration. This concentration needs first to be adjusted for exposure duration (i.e., converted to represent an equivalent continuous exposure level) as shown in Equation 4-3, with the noted caution pertaining to this type of extrapolation. Following this adjustment, the RfD_{si} may be calculated as:

$$RfD_{si}\ (mg/m^3) = NOAEL_{[ADJ]}\ (mg/m^3)/(UF \times MF) \qquad (4\text{-}13)$$

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*"Free-standing" NOELs or NOAELs are those without corresponding LOAELs. In such cases the experimental threshold region has not been determined.

Some agents may not be suitable for either chronic or subchronic RfD estimation because they act in a manner distinct from those agents whose action is concentration and/or time-dependent. An example of such compounds are those that cause occupational asthma (Chan-Yeung and Lam, 1986) or induce hypersensitivity reactions. Others include agents in which adverse effects continue to progress over a period of years.

4.2.3 Issues Requiring Further Investigation

- Development of guidance on how to compare exposure duration for subchronic animal exposures with duration for subchronic human exposures for the purpose of determining whether the criterion of "equivalent duration" is met by a particular data set
- Development of specific guidance concerning application of duration-related UFs for partial lifetime exposure development

4.3 CRITERIA FOR SPECIFYING LEVEL OF CONFIDENCE

The selection of a NOAEL or other appropriate measure of threshold response involves a process that incorporates scientific subjective judgment and statistical measures of significance. The qualitative and quantitative nature of this process results in estimated benchmark values such as the RfD associated with varying degrees of confidence that can be described as high, medium, and low. The confidence ascribed to the result is a function of both the quality of the individual study and the completeness of the supporting data base. For example, the RfD verification work group assigns confidence levels to the individual study, the data base, and the RfD. Thus, if the individual study is of excellent quality, it most likely will receive a high confidence rating, even though it may be subchronic in duration. Duration of the chosen study, as well as supporting studies and the spectrum of investigated endpoints (e.g., reproductive effects), are considered in the rating of confidence in the data base. Low confidence in the data base might be given to an excellent chosen subchronic study with few supporting studies and few endpoints examined. The confidence in the RfD then would reflect these two ratings by a rating of medium to low, indicating uncertainty (lack of confidence) and suggesting that further investigations may refine this number.

The degree of confidence in a particular laboratory animal study involves a number of parameters. These parameters include, but are not limited to, the following.

- Adequacy of study design
    - Is the route of exposure relevant to humans?
    - Were an appropriate number of animals and/or sexes used for determination of statistical significance?
    - Was the duration of exposure sufficient to allow results to be extrapolated to man under different exposure conditions?
    - Were appropriate statistical techniques applied?
    - Were the analytical techniques sufficient to adequately measure the level of the test substance in the exposure protocol, including biological media?
    - Is the animal species and strain appropriate as a surrogate for man?
    - Are the techniques for measurement of the biological endpoints scientifically sound and of sufficient sensitivity?
    - To what degree are the biological endpoints qualitatively and/or quantitatively extrapolatable to humans?
- Demonstration of dose-response relationships
    - Were sufficient exposure levels used to demonstrate the highest NOAEL for the endpoint of concern?
    - Is the shape of the dose-response curve consistent with the known pharmacokinetics of the test substance?
    - Has the dose-response curve been replicated by or is it consistent with data from other laboratories and other laboratory animal species?
- Species differences
    - Are the metabolism and pharmacokinetics in the animal species similar to those for man?

  - Is the species response consistent with that in other species?
  - Is the species from which the threshold value derived the most sensitive species?

- Other factors
  - The number of biological endpoints evaluated and associated with dose-response relationships
  - Sufficient description of exposure protocol, statistical tests, and results to make an evaluation
  - Condition of animals used in the study

The degree of confidence in a particular data base also involves a number of parameters. These parameters include, but are not limited to, the following.

- Minimum data base for high confidence in an inhalation RfD:
  - Pulmonary, two well-performed chronic inhalation studies.
  - Nonpulmonary, same as oral RfD (Table 4-4) (oral studies may be appropriate for addressing questions of potential developmental and reproductive toxicity); chronic pulmonary studies may substitute for chronic oral bioassays if they are comprehensive (i.e., examined all critical endpoints)

- Minimum data base for low confidence in RfD:
  - One inhalation subchronic bioassay (that examined lung parameters in addition to others)
  - A subchronic oral study can be used, if information on inhalation is not available, with sound professional judgment.

- Oral data should not be used in the following instances:

  (1) When groups of chemicals that are expected to have different toxicity by the two routes; for example, metals, irritants and sensitizers;

  (2) when a first-pass effect is expected by the liver, or when the pulmonary system was not adequately studied in the oral studies;

TABLE 4-4. MINIMUM DATA BASE FOR BOTH HIGH AND LOW CONFIDENCE IN THE RfD

Mammalian Data Base[a]	Confidence	Comments
1. A. Two toxicity studies in different species	High	Minimum data base for high confidence
B. One reproductive study		
C. Two developmental toxicity studies in different species		
2. 1A and 1B, as above	Medium to high	
3. Two of three studies, as above in 1A and 1B; one or two developmental toxicity studies	Medium to high	
4. Two of three studies, as above in 1A and 1B	Medium	
5. One of three studies, as above in 1A and 1B; one or two developmental toxicity studies	Medium to low	
6. One of three studies, as above in 1A and 1B	Low	Minimum data base for estimation of an RfD

[a] Composed of core minimum Office of Pesticide Programs-rated studies, or studies published in refereed journals. It is understood that adequate toxicity data in humans can form the basis of a RfD and yield high confidence in the RfD without this data base.

(3) when a pulmonary effect is established but dosimetry comparison cannot be clearly established between the two routes; and

(4) when short-term inhalation studies or in vitro studies indicate potential portal-of-entry effects at the lung, but studies themselves are not adequate for an RfD development.

- Other considerations are encouraged.

The interested reader is also referred to Pepelko and Withey (1985) and National Research Council, 1986, 1987).

The level of confidence in a particular threshold value will be higher if it is derived from human data and supported by animal data. The parameters and factors involved in the evaluation of human data are described in Section 3.1.1.

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APPENDIX A
NOVEL APPROACHES TO THE ESTIMATION OF REFERENCE DOSE (RfD)

I. INTRODUCTION*

Current methods for estimating human health risks from exposure to threshold-acting toxicants in water or food, such as those established by the U.S. Environmental Protection Agency (Federal Register, 1980; U.S. Environmental Protection Agency 1987a; Stara et al., 1981), the Food and Drug Administration (Kokoski, 1976), the National Research Council (1977, 1980) or the World Health Organization, and the Food and Agricultural Organization (Bigwood, 1973; Vettorazzi, 1977, 1980; Lu, 1983), consider only chronic or lifetime exposure to individual chemicals. These methods generally estimate a single, constant daily intake rate which is low enough to be considered safe or acceptable, referred to as an acceptable daily intake (ADI).

Two general scientific problems with this approach have been long recognized (Krewski et al., 1984), in addition to its limited usefulness (i.e., lifetime health risk assessment only). The first problem is that this method does not readily account for the number of animals used to determine the appropriate NOEL. For example, if a chemical has a NOEL based on 10 animals and a similar NOEL based on 100 animals, the risk assessor often will choose the NOEL based on the larger study because it yields greater confidence in the resulting ADI**. However, if these NOELs were for different chemicals, similar RfDs might be derived even though one would be associated with much less confidence. It might be useful if the number of animals used to determine the appropriate NOEL would in some way affect the value of the resulting RfD, in addition to the level of confidence. The second problem with the current approach is that the slope of the dose-response curve of the critical toxic effect is generally ignored in the estimation of the RfD. Many scientists have argued that this slope should in some way directly affect the resulting RfD, with steep curves presumably yielding higher values because threshold is more quickly obtained.

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*Note: Although material presented in this appendix is based upon oral data, the approaches may be applicable to the inhalation RfD methodology as well. Applications would necessarily give consideration as well to the inhalation-specific issues (e.g., dose adjustment) discussed in this document.

**Now referred to by the U.S. EPA as a Reference Dose (RfD) (U.S. Environmental Protection Agency, 1987a).

Furthermore, the current approach to noncancer risk assessment yields an RfD that is presented as a single number. As such, it reflects neither the statistical variability in the NOAEL resulting from design factors of critical studies nor the known variability in uncertainty factors used to account for deficiencies in the data base. The results of this variability is the unknown range of uncertainty in the estimate. Risk management decisions for regulation or enforcement need more quantitative information on the inherent and recognized uncertainties in this assessment.

The purpose of this text is to illustrate several revised approaches to estimate RfDs that include methods for partial lifetime assessment, methods for RfD estimation with quantal or continuous toxicity data, and methods for estimating the statistical variability of NOELs and uncertainty factors. These methods address to a degree the known scientific problems with the current approach. The development of these methods can be found in Stara and Erdreich (1984a,b); these methods also are described in Stara et al. (1985) and Stara et al. (1987), and more fully in Crump (1984), Dourson (1986), and Dourson et al. (1985, 1986, and 1987).

II. AN APPROACH TO USE ALL TOXICITY DATA AND SUPPORT PARTIAL LIFETIME RISK ASSESSMENTS

a. Proposed Approach. Health risk assessments generally require evaluation of several types of toxicity data derived from several different species, different doses, different exposure durations, varied endpoints, and varied quality. This variety often makes the health risk assessment extremely difficult. Therefore, it is valuable to have all such toxicity data displayed simultaneously, if possible.

A graphic method is presented for this purpose (see Figure A-1). After thorough evaluation of the literature, toxicity data on a particular chemical might be summarized by several variables: (1) dose rate (mg/kg/day), (2) exposure duration, and (3) ranking of effects. The basis of the proposed method is empirical observation. The toxicity data from all studies (including human) are assigned to categories of severity based on observed effects in the case of graded data, or on the statistical or biological significance in the case of quantal or continuous data. Each of the effect severity levels described above is represented by a unique symbol (Table A-1).

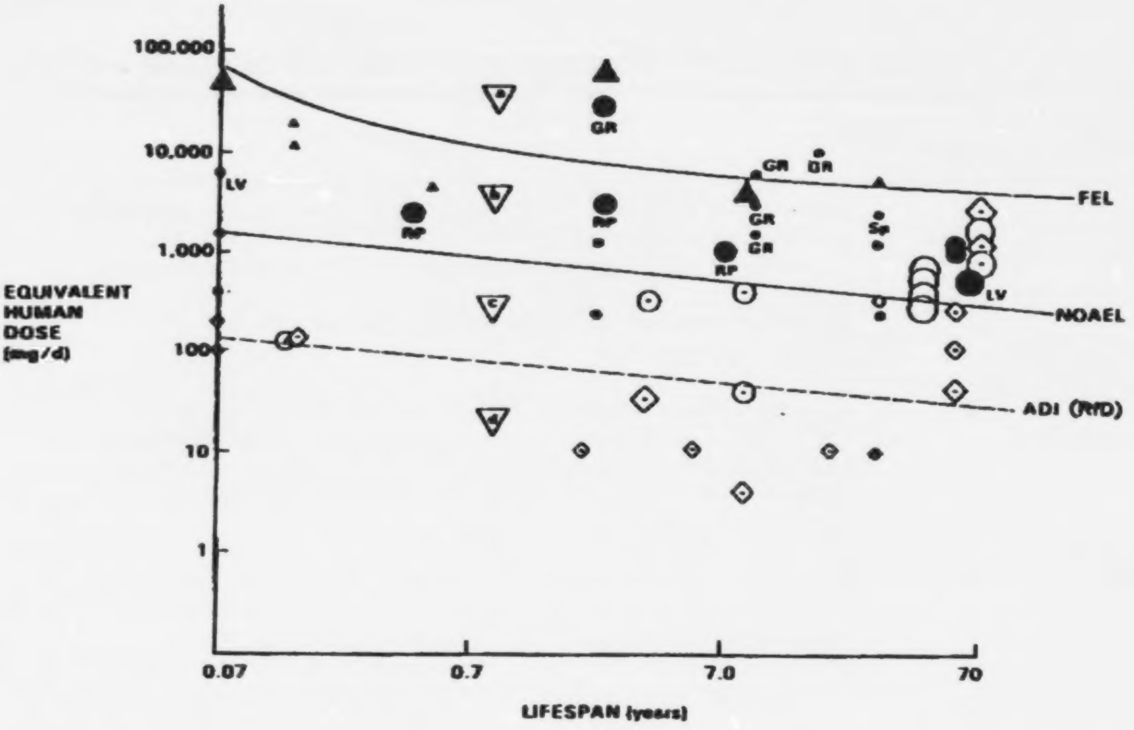

Figure A-1. Effect-dose-duration plot of all relevant human and animal oral toxicity data for methoxychlor. Effect levels indicated by symbols are defined in Table A-1. Animal doses have been converted by a body surface area factor to approximate the equivalent human dose. Dose durations are divided by the appropriate species lifespan to yield a fraction, which, when multiplied by 70 years (the assumed average human lifespan), gives the corresponding position on the x-axis. Study usefulness is denoted by symbol size. Target organs are LV (liver), RP (reproductive organ), GR (growth reduction), and SP (spleen). The dose axis is divided into areas expected to cause either (A) gross toxicity and death, (B) adverse effects, (C) nonadverse effects, or (D) no effects.

Source: Dourson (1986).

After graphic representation of all available toxicity data, a boundary line is estimated (in Figure A-1 the line has been fitted by eye) that represents for any given time the highest NOAEL for which no lower AEL is observed. Recent work by the U.S. EPA discusses statistical approaches to this boundary estimation (Hertzberg, 1989). Interpolation along this NOAEL curve can be performed to estimate the NOAEL for any desired partial-lifetime exposure. In order to obtain a corresponding acceptable intake, the estimated NOAEL could be divided by an uncertainty factor. In Figure A-1 an uncertainty factor of 100 is used and accounts for the expected intrahuman and interspecies variability

TABLE A-1. VARIOUS EFFECT LEVELS AND THEIR DEFINITIONS USED IN FIGURE A-2

Effect Level[a]	Symbol	Definition[b]
FEL	▲	Frank-Effect Level. That exposure level which produces unmistakable adverse effects, such as irreversible functional impairment or mortality, at a statistically or biologically significant increase in frequency or severity between an exposed population and its appropriate control.
AEL	●	Adverse-Effect Level. That exposure level at which there are statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.
NOAEL	○	No-Observed-Adverse-Effect Level. That exposure level at which there are no statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control. Effects are produced at this level, but they are not considered to be adverse.
NOEL	◇	No-Observed-Effect Level. That exposure level at which there are no statistically or biologically significant increases in frequency or severity of effects between exposed population and its appropriate control.

[a] Listed in order of decreasing severity.

[b] Adverse effects are considered as functional impairment or pathological lesions which may affect the performance of the whole organism, or which reduce an organism's ability to respond to an additional challenge (Federal Register, 1980).

to the toxicity of a chemical (in lieu of chemical-specific data). Both the choice of the highest NOAEL line (without lower AELs) and the suggested uncertainty factor of 100 are consistent with and a logical extension of previously established scientific principles of the U.S. EPA (Federal Register, 1980; U.S. Environmental Protection Agency, 1987a), the Food and Drug Administration (Kokoski, 1976), and the National Research Council (1977, 1980) in the use of effect levels and uncertainty factors in order to estimate ADIs or RfDs.

b. Assumptions and Limitations. The primary advantage of the graphic method is that it provides a mechanism for viewing all of the data simultaneously, resulting in an integrated profile of a compound's toxicity. In addition, exposure duration-response trends, if present, are clearly delineated, providing a possible strategy for estimating acceptable intakes for partial-lifetime exposures.

The graphical method relies on a simple severity ranking system for data presentation (for example, NOEL, NOAEL, AEL, and FEL). Obviously with such a simple system, effects within a given category (that is, all AELs) may not be identical, nor is it assumed that they are. Indeed, the critical effect is often a function of exposure duration. In these cases the effects within a given category will not be the same across time. However, the change in critical effect over duration (and, therefore, the change in effects within a category) is perhaps only of secondary regulatory importance. Since the NOAEL line is based on NOAELs of critical effects from all durations, the approach is consistent with the regulatory objective of guarding against any adverse effect. Moreover, while assumptions are needed in the process of extrapolation of dose and duration from animal studies to their human equivalent counterparts, this graphical method should enable regulatory scientists, at a glance, to judge the overall strength of evidence of toxicity and to determine data gaps wherever they appear.

One limitation of this proposed procedure is that the development of the dose rate scale does not make provisions for incorporating interspecies differences in the metabolic patterns of dealing with different chemicals; that is, the method does not take into account differences in activation and detoxification, and such. It also is assumed that the log-log plot does not overly compress the data. The problems are particularly great for very short durations of exposure. In general, the dose rate to duration ratio plots that the U.S. EPA has done so far on other chemicals have been characterized by a paucity of data for short-term exposures. Another limitation is that the time interval to develop pathologic signs after acute toxic insult may be more related to body size and pharmacokinetic parameters than a given measure of exposure duration such as days. In addition, most chemicals have scant data, and, thus, plots of these data may not yield useful generalizations.

The experiments used to develop the data base which was used to derive acceptable limiting concentrations for short durations were rarely, if ever, designed with that purpose in mind. Short-term experiments have been done in animals of many ages representing most phases of the total life span. Long-term experiments (of necessity) start with young animals and follow them through their life span. If there are age-dependent differences in the sensitivity of the experimental species, these would confound the data sets we are using.

c. Status. In summary, this novel method for estimating RfDs utilizes more of the available toxicity data than the current methodologies, and offers a consistent approach for possibly estimating health risks for less-than-lifetime toxicant exposure. A computer program facilitates use of this approach and produces the graphical display (Hertzberg, 1989). Moreover, statistical methods are being developed in order to estimate boundaries.

III. APPROACH* WITH QUANTAL OR CONTINUOUS TOXICITY DATA

a. Proposed Approach. Traditionally, NOAELs have been defined for quantal endpoints that have nonzero background incidences by choosing an experimental dose level which does not contribute to a statistically significant increase in incidence of adverse effects when compared to a control group. In parallel, NOAELs have been defined for continuous data by choosing an experimental dose level which does not constitute a significantly different mean value for a parameter, indicating an adverse effect when compared to a mean value for a control group.

As previously discussed in Section II, two limitations are inherent in this approach. The first problem is related to the insensitivity of the current method to NOELs that use different numbers of animals, 0/10 vs. 0/1,000. The second limitation is related to the general lack of use of the slope of the dose-response curve in the current approach.

The approach suggested here is not as subject to these limitations because it uses more of the dose-response or dose-effect curve. For example, an RfD might be calculated from a dose-response curve by defining an adverse effect as a risk level of more than a certain percentage above background, such as 10%. In this presentation, 10% is chosen because many of the mathematical models in current use agree well at estimated risks in this range and because the better studies have sufficient numbers of doses and animals per dose to measure this

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*This method is described in more detail by Crump (1984).

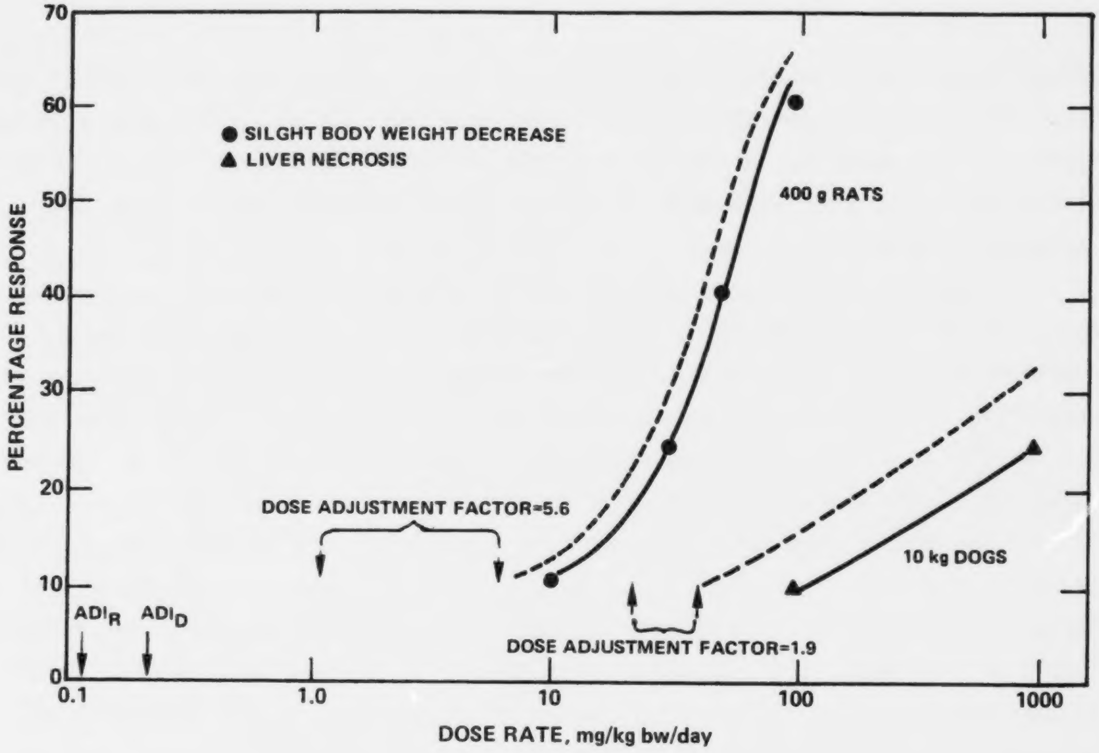

Figure A-2. Hypothetical dose-response data for slight body weight decrease (●) or liver necrosis (▲) in rats and dogs, respectively. Solid lines indicate hypothetical data; dashed lines represent lower 95% confidence limits (CLs). See text for additional explanation.

Source: Dourson (1986).

level directly. The lower 95% confidence limit (CL) on the dose associated with this risk then is calculated. In order to obtain an RfD, the dose associated with this lower 95% CL might be reduced by a chemical-specific, species adjustment factor, a tenfold uncertainty factor (this reflects the common practice), remove hyphen or as in the case of Figure A-2, the cube root of the animal body weight to human body weight ratio. Uncertainty factors might then be used to divide this adjusted value to yield the RfD.

In this presentation, uncertainty factors range between 10 and 100. The first uncertainty factor of 10 is interpreted as accounting for the expected variability in the general human population to the toxicity of the chemical. The second uncertainty factor, between 1 and 10, is thought to be necessary because the adjusted 95% CL corresponding to 10% response is considered to represent a LOAEL rather than a NOAEL. In this example, the choice for the value of this variable factor depends on both the severity of the adverse effect (i.e., more severe effects yield a larger factor) and the slope of the dose-response, or dose-effect curve (i.e., shallower slopes also yield a larger factor). For example, a choice for this variable uncertainty factor of 1 should be associated with both a minimal adverse effect and a steep dose-response or dose-effect curve.

An example of this procedure is given in Figure A-2, which is a hypothetical plot of the percentage of rats responding with a slight body weight decrease of 5% vs. dose rate or the percentage of dogs with liver necrosis vs. dose rate. Hypothetical responses are indicated by solid lines; lower 95% CLs on the dose rate are shown as dashed lines. The lower 95% CLs of the dose rates at a 10% response are adjusted by division by the cube root of the ratio of body weight between humans and rats or dogs. For rats of 400 g weight, this value is 5.6; for dogs of 10 kg weight, it is 1.9; both calculations assume a 70-kg body weight. In order to estimate RfD from the rat data (shown in Figure A-2 as ADI_R) the adjusted lower 95% CL is divided by a tenfold uncertainty factor to account for the expected variability in the general human population to the toxicity of a chemical in lieu of specific data, and an additional 1.0-fold factor because the effect is both minimally severe and has a steep dose-response slope. Thus, the total uncertainty factor is 10. In order to estimate an RfD from the dog data (shown in Figure A-2 as ADI_D) the adjusted lower 95% CL is divided by a 10-fold uncertainty factor to account for the expected human variability, as before, and an additional 10-fold uncertainty factor because the effect is more severe than a slight body weight decrease and the slope of the dose-response is shallower. Thus, the total uncertainty factor is 100.

b. Assumptions and Limitations. The proposed methods for estimating the 10% dose-effect or dose-response levels for continuous and quantal data, respectively, offer several advantages when compared with traditional methodologies (Crump, 1984). These advantages, as well as difficulties with this approach, have been discussed (Dourson et al., 1985; Crump, 1986). For example, with this new approach, both the slope of the dose-response curve and the number of animals used in an experiment can affect to some degree the estimation of the RfD when quantal or continuous toxicity data are available. Difficulties include finding appropriate data sets to model, choosing among equally good data sets that may yield different RfDs, and, for cost-benefit analysis, assuming that a certain percentage response in an animal study is equivalent to a similar percentage response in humans.

c. Status. This novel method utilizes more of the available toxicity data than the current methodology, and perhaps offers a consistent approach for possibly estimating health risks above the RfDs. It also addresses to some degree several of the criticisms of the current approach, such as use of dose-response slopes and the number of animals tested in defining NOELs. This method will be tested on a large set of toxicity data.

IV. RESEARCH ON REFINEMENTS TO THE RfD APPROACH

1. Improved Estimates of Uncertainty Factors

a. Proposed Approach. The objective of this research is to improve quantitative estimates of uncertainty factors and modifying factors used in the U.S. EPA's current approach. By evaluating the effect of deviations from the ideal desirable data base, uncertainty factors can be expressed as a range rather than as a single number. Models are being developed of the likely distribution of probability in the standard uncertainty factors.

The first step in this approach is to assemble an appropriate data base for the issue in question (i.e., which uncertainty factor is being addressed, such as the use of 10 to extrapolate subchronic to chronic data). To evaluate the standard uncertainty factors (UFs) for the RfD and to develop better estimates, it is necessary to have a relatively complete data base for a group of chemicals; for example, one that contains subchronic and chronic data and NOELs and LOAELs. Since UFs have been designed to reduce, for example, the LOAEL to a NOAEL or to reduce a subchronic NOAEL to a chronic NOAEL, the variable of interest is a ratio. This approach is to plot a frequency histogram of the ratio of the surrogate NOAEL, the LOAEL, or the subchronic NOAEL, to the best data point and fit a probability distribution to the data.

Sufficient toxicity data on sensitive populations are generally not available to test the UF for interindividual variability. However, the U.S. EPA has identified components of variability that contribute to sensitivity, and has evaluated the distribution of these pharmacokinetic parameters which determine variation in delivered dose, such as areas under the curve of blood concentration over time. Pharmacokinetic variables that affect target organ dose fit a log normal distribution. The analysis shows that values vary as much as 10-fold among normal healthy individuals (U.S. Environmental Protection Agency, 1986a); (Hattis et al., 1987).

The next step would be to model the likely overall variability in a risk estimated by means of a Monte Carlo simulation using these distributions for input. Currently, not all results fit a known probability distribution.

b. Assumptions and Limitations. This approach is designed to obtain better quantitative estimates for some assumptions currently used, such as the 10-fold UF for adjusting subchronic data to chronic. It assumes data similar to that currently used to derive RfDs.

c. Status. More data are needed to model these UFs. The data base for interindividual variability could be expanded from a pilot study. When the probability distributions for each component of uncertainty in an RfD can be approximated, it will be possible to perform a Monte Carlo simulation to indicate the overall variability in the data and to estimate the probability for the RfD given the standard UFs. Further analyses of data on the sources of variability are needed before distribution assumptions can be made.

The estimate of the range of uncertainty for the UFs is not chemical specific. This approach will convey the scientific uncertainty to risk managers more completely than does the current approach. Uncertainty/sensitivity analysis presents data in a different form from that which risk managers are accustomed to and, therefore, will require explanation of these modifications.

2. - A Statistical Procedure for Improved Estimates of the NOAEL

a. Proposed Approach. A statistical procedure has been developed that is applicable to dichotomous data (i.e., presence/absence of a response of interest), for which comparison of unadjusted response rates is valid. (Unadjusted for differences in intercurrent mortality, or other factors that could be confounded with a treatment effect.) In samples at a control, low, and high dose, the responses are assumed to be independently distributed from binomial distributions with parameters P_0, P_1, P_2, respectively. It is further assumed that $P_0 \leq P_1 \leq P_2$, and that a treatment effect, if present, increases the response rate. An important aspect of the statistical method employed here is that observed response rates are replaced by the maximum likelihood estimates of P_0, P_1, and P_2.

The procedure estimates the maximum likelihood for all doses and estimates the standard deviation of the NOAEL estimate. It also estimates, for each experimental dose, the probability of getting the observed result under the hypothesis of "no treatment effect." Thus, the NOAEL can be expressed as a range. The power of the test is a function of background rate, with lower backgrounds yielding higher power. The test characteristics are discussed in U.S. Environmental Protection Agency (1988a).

The following example demonstrates the type of results obtained from this procedure. In a study using a control and doses of 30 and 100 mg/m^3, the procedure rejects the hypothesis of no treatment effect at the high dose ($p \leq 0.04$). The expected value of the NOAEL is 47 mg/m^3, and the bounds at one standard deviation are 17 and 77 mg/m^3. The probability of obtaining the observed response under the null hypothesis is 76% at 30 mg/m^3 and 24% at 100 ppm. In comparison, under the existing risk assessment procedure, the study would provide only a NOAEL of 30 mg/m^3.

The response probabilities express the level of certainty of confidence in the data. The range of one standard deviation could easily be expressed in the RfD simply by applying UFs to upper and lower limits of the estimate.

b. Assumptions and Limitations. This procedure is designed for dichotomous (incidence) data and is a sequential test appropriate for three dose groups. While initially designed for three doses and sample sizes up to 20, it has the capacity to be extended for more dose groups and larger sample sizes. It assumed that a treatment effect, if present, increases the response rate, and that responses are to be independently distributed from binomial distributions.

c. Status. The document describing the method developed (U.S. Environmental Protection Agency 1988a) has been reviewed by U.S. EPA statisticians and revised according to these comments. The procedure has been presented at two scientific meetings. A computer program is available for easy implementation of the procedure on PCs.

APPENDIX B
USE OF PHARMACOKINETIC DATA IN RISK ASSESSMENT, SELECTED EXAMPLES

While the U.S. Environmental Protection Agency has had little experience in the development of inhalation reference doses, potency estimates for inhalation exposure to carcinogens have been developed for quite some time. Examples of the way that the Agency has utilized pharmacokinetic data to adjust dose estimates for carcinogens illustrate both the necessity for utilizing all available pharmacokinetic data, as well as the kind of empirical adjustments which can be made to dose estimates, even in situations where complex physiologically based pharmacokinetic modeling is not feasible.

Example 1: Nonlinear absorption with increasing air concentration.

This example is taken from a U.S. Environmental Protection Agency publication (1985) which discusses the carcinogenicity of butadiene. The retained dose vs. exposure concentration data that were developed separately from the carcinogenicity evaluation are shown in Table B-1.

TABLE B-1. ABSORPTION OF 1,3-BUTADIENE BY INHALATION FOLLOWING A 6-HOUR EXPOSURE PERIOD

Species	Exposure (ppm)	(μg/ℓ)	1,3-Butadiene inhaled (μmol)	1,3-Butadiene retained (μmol)	(μmol/kg)	Percent Retained
Rats	70	125	235	16.3	40	7.1
	930	1,700	3,100	64.7	160	3.1
	7,100	12,800	17,000	243.0	660	1.5
Mice	7	13	1.7	0.9	33	54.0
	80	145	34.7	3.2	120	9.6
	1,040	1,900	435.0	19.1	660	4.7

The actual exposure concentrations in the cancer bioassays were 625 ppm and 1,250 ppm for mice, and 1,000 ppm and 8,000 ppm for rats. By graphing log ppm exposure vs. log-retained dose from the pharmacokinetic study, the U.S. Environmental Protection Agency (1985) estimated the retained doses for each of the experimental exposure concentrations used in the cancer bioassay; that is 25.7 and 38.9 mg/kg retained dose for mice, and 10.5 and 37.1 mg/kg for rats. After developing a unit risk estimate based on the relationship between retained dose and tumor incidence, the unit risk was converted back into units of air concentration by making an assumption concerning percent retention by humans at low exposure concentrations. If a model that assumed that retained dose was proportional to exposure concentration were assumed, the data would have suggested a greater than 100-fold difference in retained dose from low dose to high dose in the rat study, when in fact only a 5-fold difference was apparent, based upon retained dose estimates. Similarly, a dose proportional to concentration assumption for mice would have suggested a 150-fold difference between low and high dose while the retained dose fraction suggests only an 11-fold difference.

The significance of this for inhalation RfD estimation is considerable, especially in situations where an RfD might be derived based upon a LOAEL. For example, if we theoretically had a single exposure concentration inhalation study of butadiene which provided data indicating that 1,040 ppm was a LOAEL, the following situation could be envisioned. If a dose proportional to concentration model is assumed, either based upon computing dose utilizing ventilatory volume or using a metabolic rate estimate, the following scenario could be envisioned:

$$1{,}040 \text{ ppm} = 1{,}900 \text{ mg/m}^3$$

> 1,900 mg/m^3 x 0.01 m^3/day (mouse ventilatory volume for 6 hours) ÷ 0.03 kg (mouse body weight) ÷ UF of 1,000 (10 LOAEL to NOAEL, 10 for interspecies, 10 for sensitive subgroups) = 6.3 mg/kg/day x 70 kg ÷ 20 m^3 = 2.22 mg/m^3 as the reference air concentration for 24-hour human exposure.

In contrast, using the retention data, the mouse exposure concentration corresponding to a 10-fold lower retained dose (estimated from data in Table B-1) is 45.9 mg/m^3. This would be equivalent to estimating a NOAEL exposure level for the mouse based upon retained dose:

> 45.9 mg/m^3 x 0.01 m^3/6 hours ÷ 0.03 kg ÷ 100 UF (10 for interspecies, 10 for intraspecies) = .11 mg/kg/day x 70 kg ÷ 0.5 (estimate of human retained dose at low concentrations) ÷ 20 m^3 = 1.07 mg/m^3 as the reference air concentration for 24-hour human exposure.

This represents a twofold difference which would be essentially equivalent to reducing the UF for extrapolating from a LOAEL from 10 to 5. This example assumes that a steady state is reached within the 6-hour exposure period. If this is not the case, linear extrapolation to a 24-hour exposure period would be inappropriate.

Example 2: Metabolic Saturation at High Exposure Concentrations

Since animal bioassays are traditionally conducted at high exposure concentrations and the results extrapolated to lower exposure concentrations, the issue of saturable metabolic capacity is relevant. This consideration is equally appropriate to both the oral and inhalation exposure routes. While the impact of capacity-limited metabolism may be of greater concern for carcinogen exposures where a linear, nonthreshold dose-response curve is assumed and risks resulting from human exposures to very small quantities of the chemical of concern are extrapolated from high dose or concentration animal exposures, a potential for impact in the assessment of noncancer endpoints still exists. Typically, an RfD is developed by applying a composite uncertainty factor of from 100 to up to 10,000, to an exposure level or dose which has been experimentally evaluated in an animal test system. If the critical effect is the result of the interaction of a metabolite with the target tissue, and if nonlinearity in the metabolized fraction of the dose exists within the range of doses or exposure concentrations encompassed by the difference between the experimentally evaluated dose and the projected RfD exposure level, the actual difference between the experimental and extrapolated dose will be less than that predicted, based upon a linear relationship between exposure and effective dose to the target tissue. The result of this could be interpreted as an effective erosion of the magnitude of the composite uncertainty factor. On the other hand, if good pharmacokinetic data are available for both the experimental animal system and the human, it may be feasible to reduce the magnitude of the uncertainty factor.

An impediment to the use of pharmacokinetic data for the adjustment of animal dose response data in evaluations of noncancer endpoints is that the chemical species resulting in the critical effect is less frequently identified than for carcinogenic responses. However, it is still appropriate to evaluate all of the available pharmacokinetic data for potential relevance to the RfD derivation exercise. This will become increasingly important as the Agency moves from single medium, single route assessments towards methods for effectively partitioning RfDs across media/routes.

The following example is taken from U.S. EPA (1986e). In this assessment, unit risk estimates were developed for human exposure to low levels of tetrachloroethylene by first developing animal dose-response relationships based upon the extrapolated animal metabolized dose at each inhalation exposure concentration.

Table B-2 illustrates that while the total radioactivity recovered in the 72 hours following exposure of rats for a 6-hour time interval to two concentrations of ^{14}C-tetrachloroethylene showed linearity between total recovered radioactivity and exposure concentration, there was nonlinearity in the fraction of the radioactivity attributed to metabolism.

TABLE B-2. RECOVERY OF ^{14}C-TETRACHLOROETHYLENE RADIOACTIVITY AFTER INHALATION EXPOSURE FOR 6 HOURS TO SPRAGUE-DAWLEY RATS

	10 ppm	600 ppm
	mg-equivalent tetrachloroethylene	
Expired Unchanged	1.008 (68%)	68.39 (88%)
Metabolized	0.467 (32%)	9.11 (12%)
Total	1.475	77.5

APPENDIX C

ADVERSE HUMAN RESPIRATORY HEALTH EFFECTS*

These criteria were developed to assist in the interpretations of the epidemiologic literature on what constitutes an adverse respiratory health effect of air pollution. Adverse human health effects caused by air pollution are listed in hierarchical order, with the most severe at the top and the least severe at the bottom. The reader is referred to the American Thoracic Society (1985) guidelines for more detailed discussion.

1. Increased mortality. (Increased as used here and subsequently means significantly ($p < 0.05$) increased above that recorded in some standard, comparable population. In selected situations, $p < 0.1$ may be appropriate.)
2. Increased incidence of cancer.
3. Increased frequency of symptomatic asthmatic attacks.
4. Increased incidence of lower respiratory tract infections.
5. Increased exacerbations of disease in humans with chronic cardiopulmonary or other disease which could be reflected in a variety of ways, including the following:
    - Less able to cope with daily activities (i.e., shortness of breath or increased anginal episodes).
    - Increased hospitalizations, both frequency and duration.
    - Increased emergency ward or physician visits.
    - Increased pulmonary medication.
    - Decreased pulmonary function.
6. Reduction in forced expiratory volume at one second (FEV_1) or forced vital capacity (FVC) or other tests of pulmonary function such as the following:
    - Chronic reduction in FEV_1 or FVC associated with clinical symptoms.

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*Source: American Thoracic Society, 1985.

- A significant increase in number of persons with FEV_1 below normal limits; chronically reduced FEV_1 is a predictor of increased risk of mortality. Transient or reversible reductions that are not associated with an asthmatic attack appear to be less important. It should be emphasized that a small but statistically significant reduction in a population mean FEV_1 or $FEV_{0.75}$ is probably medically significant to them, but when diluted with the rest of the population, the change appears to be small.

- An increased rate of decline in pulmonary function (FEV_1), relative to predicted value in adults with increasing age or failure of children to maintain their predicted FEV_1 growth-curve. Such data must be standardized for sex, race, height, and other demographic and anthropometric factors.

7. Increased prevalence of wheezing in the chest, apart from colds, or of wheezing most days or nights. (The significance of wheezing with colds needs more study and evaluation.)
8. Increased prevalence or incidence of chest tightness.
9. Increased prevalence or incidence of cough/phlegm production requiring medical attention.
10. Increased incidence of acute upper respiratory tract infections that interfere with normal activity.
11. Acute upper respiratory tract infections that do not interfere with normal activity.
12. Eye, nose, and throat irritation that may interfere with normal activity (i.e., driving a car) if severe.
13. Detection of odors.

APPENDIX D

CRITERIA FOR ASSESSING THE QUALITY OF INDIVIDUAL EPIDEMIOLOGICAL STUDIES*

A minimally acceptable study should meet the following criteria, which fundamentally represent good scientific practice. The study should have been reported or should be in press in the peer-reviewed literature.

1. The pertinent scientific background, such as reviews and supporting rationale upon which the study was based, should be given. Sponsorship and funding sources should be acknowledged.
2. The objectives of the study should be clearly stated and the study design described in relation to them. Underlying assumptions and limitations of the design also should be given.
3. The study population and comparison group description should include the specific population from which they were drawn and the method of selection. The rationale and criteria for inclusion/exclusion in the study should be given, particularly for exposure classifications. The appropriateness and limitations of the comparison group should be discussed. The extent to which the choice of subjects depended on existing or specially developed record systems, and implications of this upon the analysis, should be considered. The steps taken to ensure confidentiality of the subjects should be accounted for.
4. Methods of data collection should be described in detail, since these procedures will influence the derived interpretation and inferences. The validity (accuracy) and reliability (reproducibility) of the methods used to determine exposure should be stated. Response rates, including reasons for implications of differing rates, should be given. The direction and possible magnitude of any bias introduced into the study as a result of these rates should be described. The procedures used for following the study, methods to ensure completeness, and length of follow-up for each group or subgroup must be included. Other validity checks (e.g., avoiding bias by the independent ascertainment and classification of study variables, such as blind reading of histologic slides or clerical processing of data) also should be included.

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*Adapted from: Interagency Regulatory Liason Group, 1981; Lebowitz, 1983; American Thoracic Society, 1985.

5. Major demographic and anthropometric confounding factors should have been accounted for, such as age, sex, ethnic group, socioeconomic status, smoking status, and occupational exposure. Temperature, season, and day of the week are particularly important for acute studies of respiratory effects and also should be accounted for.
6. The procedures and statistical methods used to describe the data, estimate parameters, or test specific hypotheses should be presented. References and/or specific formulae also should be given for the statistical tests and for any programming procedures or packages that were applied.

The underlying assumptions and potential bias of the statistical methods should be stated. Explicit description of any method used to account for confounding factors (e.g., adjustment or matching) should be described explicitly. This includes methods to account for missing data, such as from nonresponse, attrition, or loss-to-follow-up. When reporting hypothesis tests, the measure of effect, statistical significance, power, and other criteria (e.g., one- vs. two-tailed test rationale) should be given. The point estimates and their standard errors and/or confidence intervals should be given when using estimation.

APPENDIX E

CRITERIA FOR ASSESSING THE QUALITY OF INDIVIDUAL ANIMAL TOXICITY STUDIES*

A minimally acceptable study should meet the following criteria, which fundamentally represent good scientific practice.

1. All elements of exposure should be clearly defined.

   - The exposure amount, administration route, exposure schedule, and exposure duration must be described. Consideration should also be given to the concentration and time of exposure used vs. the expected level of human exposure.
   - If animal body weights, ages, or gender are not provided, consideration should be given to the uncertainty in appropriate default values.
   - Exposure information should include physicochemical characteristics of the substance used, such as purity, stability, pH, partition coefficient, particle size distribution, and vehicle. These properties can influence the local effects and the rate and extent of absorption, which can subsequently modify the toxic manifestations.
   - Exposure information should include description of generation and characterization technology used. The number of air changes, temperature, and relative humidity are exposure chamber characteristics which should be monitored. Cage (or other animal holder) rotation schedule should be described.
   - Animal care and holding procedures should be described.

2. Controls should be comparable with test animals in all respects except the treatment variable ("negative").

   - Concurrent controls must minimally include an "air-only" exposure group; if a vehicle is used, it is desirable that there be a "vehicle-only" group.
   - Historical control data can be useful in the evaluation of results, particularly where the differences between control and treated animals are small and are within anticipated incidences based on examination of historical control data.

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*Adapted from: Society of Toxicology, 1982; Muller et al., 1984; National Research Council, 1984; James, 1985; and Lu, 1985a.

3. Endpoints should answer the specific hypothesis addressed in the study, and the observed effects should be sufficient in number or degree (severity) to establish a dose-response relationship that can be used in estimating the hazard to the target species.

   - The outcome of the reported experiment should be dependent on the test conditions and not influenced by competing toxicities.

4. The test performed must be valid and relevant to human extrapolation. The validity of using the test to mimic human responses must always be assessed. Issues to consider include the following:

   - Does the test measure a toxicity directly or does it measure a response purported to indicate an eventual change (i.e., severity of the lesion)?
   - Does the test indicate causality or merely suggest a chance correlation?
   - Was an unproven or unvalidated procedure used?
   - Is the test considered more or less reliable than other tests for that endpoint?
   - Is the species a relevant or reliable human surrogate? If this test conflicts with data in other species, can a reason for the discrepancy be discerned?
   - How reliable is high exposure (animal) data for extrapolation to low exposure (human scenario)?

5. Conclusions from the experiment should be justified by the data included in the report and consistent with the current scientific understanding of the test, the area of toxicology being tested, and the suspected mechanism of toxic action.

6. Due consideration in both the design and the interpretation of studies must be given for appropriate statistical analysis of the data.

   - Statistical tests for significance can be performed only on those experimental units that have been randomized (some exceptions include weight-matching) among the dosed and concurrent control groups.

- Some frequent violations of statistical assumptions in toxicity testing include:
  - Lack of independence of observations.
  - Assuming a higher level of measurement than available (e.g., interval rather than ordinal).
  - Inappropriate type of distribution assumed.
  - Faulty specification of model (i.e. linear rather than nonlinear).
  - Heterogeneity of variance or covariance.
  - Large Type II error.

7. Subjective elements in scoring should be minimized. Quantitative grading of an effect should be used whenever possible.

8. Evidence of adherence to good laboratory practices is required unless exceptions have been negotiated (current testing) or considered (data obtained from studies carried out many years ago). See also Section 3.1.2.3.
9. Peer review of scientific papers and of reports is extremely desirable and increases confidence in the adequacy of the work.
10. Reported results have increased credibility if they are reproduced by other researchers and supported by findings in other investigations.
11. Similarity of results to those of tests conducted on structurally related compounds should be considered.

APPENDIX F

CRITERIA FOR CAUSAL SIGNIFICANCE

Statistical methods cannot establish proof of a causal relationship but can define an association with a certain probability. The causal significance of an association is a matter of judgment that goes beyond any statement of statistical probability. To assess the causal significance of an air toxicant and a health effect, a number of criteria must be used, no one of which is pathognomonic by itself. These criteria include the following:

- Consistency (reproducibility) of the association. Causal inferences are strengthened when a variety of investigators have reproduced the findings under a variety of circumstances.
- Strength of the association. The larger the calculated relative risk, the greater the likelihood that the observed association is causal.
- Specificity of the association. Causality is more likely if a particular exposure is associated with only one illness and vice versa. This guideline rarely applies to air pollution research, in which all the diseases of major concern are multifactorial.
- Temporal relationship of the association.
- Coherence of the association. An epidemiologic inference of causality is greatly strengthed when it conforms to knowledge concerning the biologic behavior of a toxin and its mechanism of action. This evidence may be obtained from clinical research or toxicologic studies.

APPENDIX G

CHOICE OF TOXICITY DATA*

Empirical observation generally reveals that as the dosage of a toxicant is increased, the toxic response (in terms of severity and/or incidence of effect) also increases. This dose-response relationship is well-founded in the theory and practice of toxicology and pharmacology. Such behavior is observed in: (1) quantal responses, in which the proportion of responding individuals in a population increases with dose; (2) graded responses, in which the severity of the toxic response within an individual increases with dose; and (3) continuous responses, in which changes in a biological parameter (e.g., body or organ weight) vary with dose.

However, in evaluating a dose-response relationship, certain difficulties arise. For example, one must decide on the critical endpoint to measure as the response. One also must decide on the correct measure of dose. In addition to the interspecies extrapolation aspects of the question of the appropriate units for dose, the more fundamental question of administered dose vs. absorbed dose vs. target organ dose should be considered. These questions are the subject of much current research.

1. Critical Study and Species. Often animal data are selected as the governing information for quantitative risk assessments, since human data are generally either unavailable or insufficient for this purpose. These animal studies typically reflect situations in which exposure to the toxicant has been carefully controlled, and the problems of heterogeneity of the exposed population and concurrent exposures to other toxicants have been minimized. In evaluating animal data, a series of professional judgments are made that involve, among others, consideration of the scientific quality of the studies. Presented with data from several animal studies, the risk assessor first seeks to identify the animal model that is most relevant to humans, based on the most defensible biological rationale; for instance, using comparative pharmacokinetic data. In the absence of a clearly most relevant species, however, the most sensitive

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*Adapted from U.S. Environmental Protection Agency, 1987a.

species (i.e, the species showing a toxic effect at the lowest administered dose) is adopted as a matter of science policy at EPA, since no assurance exists that humans are not innately more sensitive than any species tested. This selection process is made more difficult if animal tests have been conducted using different routes of exposure, particularly if the routes are different from those involved in the human situation under investigation.

In any event, the use of data from carefully controlled studies of genetically homogeneous animals inescapably confronts the risk assessor with the problems of extrapolating between species, and the need to account for human heterogeneity and concurrent human exposures to other chemicals, which may modify the human risk.

While there has generally been a lack of well-controlled cohort studies that investigate noncancer endpoints and human exposure to chemicals of interest by the oral exposure route (a useful exception being the cases of cholinesterase inhibition), it is anticipated that there will be considerably more human data which may be selected as the critical data for inhalation exposure assessments. Risk assessments based on human data have the advantage of avoiding the problems inherent in interspecies extrapolation. In many instances, as is the case with the animal investigations, use of such studies involves extrapolation from relatively high doses and relatively healthy populations (such as those found in occupational settings) to the low doses found in the environmental situations to which the general population is more likely to be exposed. In some cases, a well-designed and well-conducted epidemiologic study that shows no association between known exposures and toxicity can be used to directly project an RfD, as has been done in the case of oral exposure to fluoride (U.S. Environmental Protection Agency, 1986d).

2. Critical Data. In the simplest terms, an experimental exposure level is selected from the critical study that represents the highest level tested in which the critical toxic effect was not demonstrated. Where appropriate, adjustments in doses based upon known interspecies differences in respiratory tract deposition must be applied before arraying the dose-effect data to compare species sensitivity. This NOAEL is the key datum gleaned from the study of the dose-response relationship and, traditionally, is the primary basis for the scientific evaluation of the risk

posed to humans by systemic toxicants. This approach is based on the assumption that if the critical toxic effect is prevented, then all toxic effects are prevented.

3. Dosimetric Adjustments. Exposure effect levels observed in animal studies of any given data array on a chemical must be converted to human equivalent concentrations before comparisons of species sensitivity and the choice of the appropriate animal effect and critical study can be made. Conversions to human equivalent concentrations are made by applying adjustment factors to account for dosimetric differences of agents (particles or gases) between individual animal species and humans, as discussed in Chapter 4 and Appendices H and I.

4. Examples of "Appropriate" Choice. In the course of many risk assessment discussions during the last several years, the Agency has decided on the following conditions in choosing the appropriate animal effect or no-effect level as a basis of an RfD. If an appropriate human study with a well-defined NOAEL is available as to a chemical's critical effect, it is used in preference to animal toxicity data in estimating RfDs. When such human data are not available, the following sequence is used to choose the appropriate study, species and NOAEL as a basis of RfD estimation. It should be noted that this choice should be based on human equivalent concentrations, that is, concentrations adjusted for dosimetric differences between animals and humans as described in Chapter 4.

   - The Agency chooses the most appropriate NOAEL of the critical effect from a well-conducted study on a species that is known to resemble the human in response to this particular chemical (e.g., by comparative pharmacokinetics).

   - When the above condition is not met, the Agency generally chooses the most sensitive study, species, and NOAEL, as judged by an interspecies comparison of the NOAEL and LOAEL. Table G-1 outlines examples of this condition.

TABLE G-1. COMPARISON OF THE HIGHEST INDIVIDUAL SPECIES HUMAN EQUIVALENT* NOAEL AND ITS LOAEL (OR LEL)

Effect Level (mg/m^3)	Species			Comments
	Dog	Rat	Mouse	(Given The Same Critical Effect)
Example 1:				
LOAEL (LEL)	100	120	-	The proper choice is generally the highest dog NOAEL of 50 mg/m^3, since the potential experimental threshold in dogs (i.e., the potential LOAEL) may be below the highest NOAELs in both rats and mice.
NOAEL	50	60	80	
Example 2:				
LOAEL (LEL)	120	100	90	The proper choice is generally the mouse LOAEL (or LEL) of 90 mg/m^3, since the potential experimental threshold in mice may be less than the highest NOAELs for both dogs and rats. Judgment is needed in this example to ensure that the adverse effects seen in all three species are truly minimal. For example, if any of the LOAELs (or LELs) in the species represented an increase in mortality, no firm basis for the development of an RfD exists. This is based on the general observation that mortality data are far removed quantitatively from chronic LOAELs and NOAELs, and thus, the data base has failed to establish the likely experimental threshold for the most sensitive endpoint.
NOAEL	90	75	-	
Example 3:				
LOAEL (LEL)	75	80	90	The proper choice is generally the dog LOAEL of 75 mg/m^3, since by definition this represents the most sensitive species (see, however, the caution in Example 2).
NOAEL	-	-	-	

(continued on the following page)

TABLE G-1. COMPARISON OF THE HIGHEST INDIVIDUAL SPECIES HUMAN EQUIVALENT* NOAEL AND ITS LOAEL (OR LEL) (continued)

Effect Level (mg/m^3)	Species Dog	 Rat	 Mouse	Comments (Given The Same Critical Effect)
Example 4:				
LOAEL (LEL)	-	-	-	The proper choice is generally the highest rat NOAEL of 90 mg/m^3, since no assurance exists that the experimental threshold in rats is not below the highest NOAELs of both dogs and mice. This situation is unusual and should be judged carefully; since a LOAEL (or LEL) has not been determined, the RfD may be unduly conservative. Strict interpretation of this example might lead to strikingly lower RfDs if other species are tested at much lower doses. Such RfDs may not be appropriate.
NOAEL	100	90	120	

*Human equivalent NOAEL or LOAEL refers to concentrations adjusted for dosimetric differences between animals and humans.

APPENDIX H

CALCULATION OF RDDR AND AN EXAMPLE APPLICATION OF DOSIMETRIC ADJUSTMENT FOR PARTICLE EXPOSURES

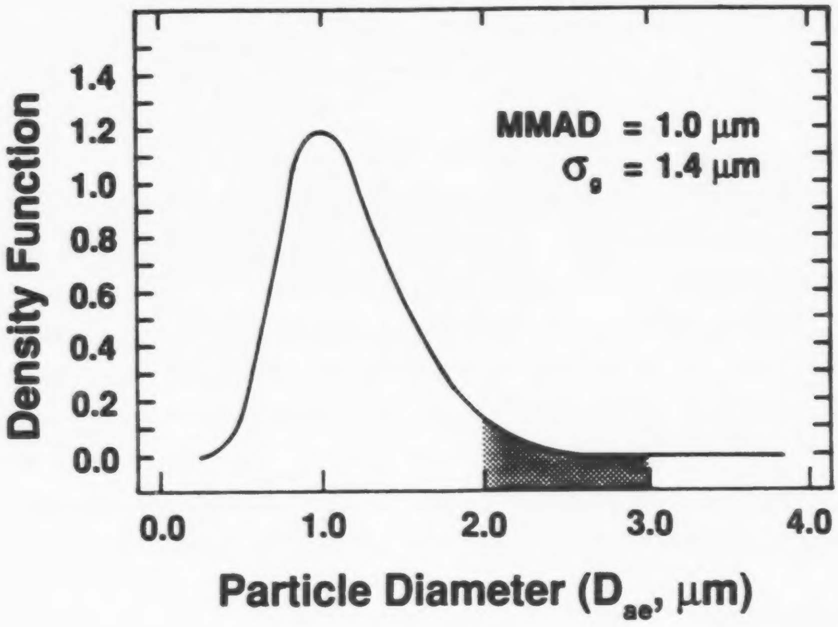
INTRODUCTION

The purpose of this appendix is to illustrate how the Regional Deposited Dose Ratio (RDDR) is calculated for use in the adjustment of exposure effect levels for dosimetric differences between species as in Section 4.1.1.2.3. Further refinement of this adjustment, as recommended by the external workshop review committee, is described in the research and development section at the end of this Appendix. The adjustment of exposure effect levels in rats for the theoretical compound ep(a)oxide will be used to illustrate this application. The health effects data shown for the compound ep(a)oxide are motivated by actual data on the toxicological effects of various aerosols.

METHODS

The initial regional respiratory tract deposition of a given aerosol exposure to an experimental species can be calculated using typical aerosol distribution data (i.e., an aerosol characterized by a given mass median aerodynamic diameter (MMAD) and a geometric standard deviation [σ_g]). The Regional Deposited Dose (RDD), or mass of aerosol initially deposited, for a given species is generated by integrating the product of an aerosol distribution and the deposition efficiency curves in regions (extrathoracic, tracheobronchial, and pulmonary) of the lung. A schematic of this integration is shown in Figure H-1 for the rat. The area under the particle distribution curve of each particle size diameter interval, for example, the interval of 2-3 µm (grey shading), is integrated with the deposition efficiency curve of a particular lung region for that same interval. Summation of these products across all the particle size ranges yields the RDD to that region (computed notationally in Equation 4-4). The RDD is calculated for each region of the lung; that is the extrathoracic (ET), region the tracheobronchial (TB) region, the pulmonary (PU), region the thoracic (TH), region and the total respiratory (TOT) system. These estimates are then adjusted for ventilatory parameters and lung surface areas.

A. Aerosol Distribution



B. Deposition Efficiency (Rat)

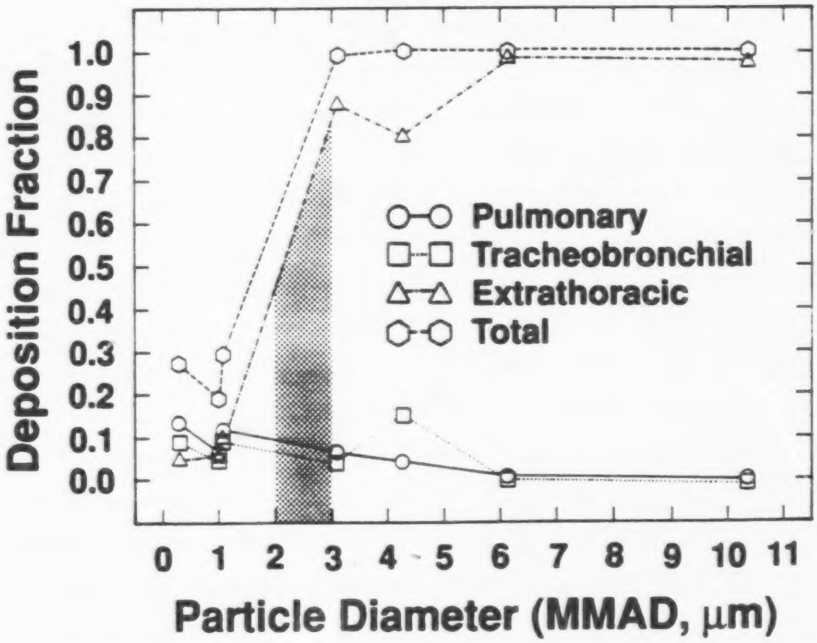


Figure H-1. Schematic of the integration of aerosol distribution (A) and deposition efficiency (B) curves for calculation of (RDD).

Source: Jarabek et al. (1989a)

The rat data used in this presentation for RDD and RDDR calculations (Jarabek et al., 1989a) are those of Raabe et al. (1988). The ET deposition deposition was calculated as the sum of the laryngeal, nasopharyngeal, and gastrointestinal fractions reported. These data were reported as means so that it was not possible to fit nonlinear regression models as was done for the humans. RDDs were estimated by linear interpolation instead.

The human RDD values were calculated similarly to calculations for the rat. Extrathoracic deposition was estimated as a function of ($\rho d^2 Q$) where ρ is particle mass density (g/m^3), d is the geometric particle diameter (μm), and Q is the airflow rate (cm^3/sec). Equations were estimated separately for experiments in which nasal breathing or oral breathing was used (Miller et al. 1988). Extrathoracic deposition then was calculated for normal augmenters (people who habitually breathe through the nose except in exercise conditions) and for mouth breathers using a proportionality factor for the split in airflow between nose and mouth as given in Niinimaa et al., (1981). Logistic regression models were used to estimate the human TB region deposition as a function of aerodynamic diameter. The models used were those developed by Miller et al. (1988), based on percentage of particles entering the trachea and were fit to TB deposition from several laboratories. The PU region deposition estimates for humans were calculated based on a theoretical model presented in Martonen and Miller (1986).

The surface area value of the ET region for the rat was calculated from the length and perimeter data in Schreider and Raabe (1981). For humans, the ET region surface area value was estimated by representing the region as sequential cylinders, using empirical data for volume and length values from solid silicone casts (Patra et al. 1986). The "whole" lung model of Yeh et al. (1979) was used to estimate the surface-area values for the TB and PU regions of the rat. The human data of Weibel (1963) on the number of dimensions of airways (represented as cylinders) in each generation were modified in a manner similar to that of Paiva (1973) to estimate the human surface-area values for the TB and PU regions (Miller et al. 1985). The procedure used to adjust the airway dimensions of the TB and PU from total lung capacity to function residual capacity (FRC = 50% TLC) is described in Overton et al. (1987). The minute volume reported by Raabe et al. (1988) was used for the rat. The default value used by the U.S. EPA, 20 m^3/day (13.8 ℓ/min), was used for the human value.

It is recognized that this approach is based on deposition efficiency data obtained or derived under a particular set of ventilatory parameters; that is, the experimental parameters for the animal and a derived human breathing pattern (13.8 ℓ/min or 20 m^3/day). The assumption in this application is that it is valid to linearly extrapolate from these values to other sets of breathing parameters. The parameters of this assumption, such as the effect of activity pattern and allometric relationships between lung weight, lung surface area, minute volume, and body weight (Adolph, 1949; Weibel, 1972; U.S. Environmental Protection Agency, 1988c) remain to be investigated as part of this methodology development.

The RDD for the species in question then can be divided by the corresponding RDD for humans to calculate the relative ratio of deposition in that species to the deposition in humans. That is, the Regional Deposited Dose Ratio (RDDR) then is calculated by:

$$\text{RDDR} = (\text{RDD})_A/(\text{RDD})_H$$

where: $(\text{RDD})_A$ = regional deposited dose in species of interest, adjusted for surface area and ventilatory volumes, and

$(\text{RDD})_H$ = regional deposited dose in humans, adjusted for surface area and ventilatory volumes.

The appropriate RDDR to calculate is dictated by the observed toxicologic effect. For example, the RDDR for extrarespiratory (ER) effects (RDDR_{ER}) would be computed (Equation 4-6, 4-7) to determine the dose to the respiratory system in order to assess an ER toxic effect (i.e., the assumed default until clearance, uptake, metabolism, and distribution functions are incorporated). However, the RDDR for the TB region alone (RDD_{TB}) would be calculated for an effect involving conducting airways, and the RDDR_{PU} for an effect involving the PU region. An effect involving the entire respiratory system would be correct by RDDR_{TOT}.

It should be noted that for "lung" (TH) effects, the appropriate RDDR to use for adjustment is the RDDR for the TB and PU regions together. The RDDR values for the TB and PU regions cannot be added together as they appear in Table H-1, however, due to the surface area and ventilatory parameter corrections to the respective deposited dose of each. Therefore, a TH column has been provided which includes the appropriate calculations.

The RDDR then can be used to scale the exposure concentration associated with the observed effect to an equivalent concentration which reflects dosimetric differences between humans and the experimental species in question. That is, the RDDR provides a factor for adjusting the no observed adverse effects level (NOAEL), according to Equation 4-5 for respiratory tract effects:

$$\text{NOAEL}_{[\text{HEC}]}\ (\text{mg/m}^3) = \text{NOAEL}_{[\text{ADJ}]}\ (\text{mg/m}^3) \times \text{RDDR}_{(\text{ET, TB, PU, TH or TOT})}$$

where: $\text{NOAEL}_{[\text{ADJ}]}$ = the NOAEL adjusted for duration according to Equation 4-3, and

RDDR = $(\text{RDD})_A/(\text{RDD})_H$, the ratio of regional dose in animal species to that of humans across regions of interest for the toxicologic effect.

This is the NOAEL level that then would be arrayed with other NOAELS to determine the most sensitive species and the key study as described in Appendix D. RDDR values for the rat to the human deposition are provided in Table H-1. As mentioned, the $\text{RDDR}_{(\text{ER})}$ is computed to adjust for ER effects. Equation 4-6 is used to calculate the RDD expressed as mg/kg per minute:

$$\text{RDD}_{\text{ER}} = \frac{10^{-6}\ YV_Tf}{\text{BW}} \sum^{n} P_i\ E_i$$

where:

P_i = the particulate mass fraction in the exposure size distribution (MMAD, σ_g),

E_i = the deposition efficiency of that size distribution (MMAD, σ_g) in the entire respiratory tract for the species of interest,

n = number of size ranges,

Y = exposure level (mg/m³),

V_t = tidal volume (mℓ),

f = breathing frequency (breaths/min), and

BW = body weight (kg).

TABLE H-1. RDDR VALUES BY MASS MEDIAN DIAMETER AND STANDARD DEVIATION FOR RATS*

Sigma g	MMAD	ET	TB	PU	TH	TOT	ER
1.200	0.100	1.5195	H	0.6385	1.1165	1.7661	0.0096
1.200	0.200	0.4432	61.8242	1.1253	1.9483	2.5931	0.0141
1.200	0.300	0.2263	20.6081	1.5359	2.5809	2.6349	0.0143
1.200	0.400	0.1437	12.3648	1.7485	2.8390	2.2689	0.0123
1.200	0.500	0.1023	8.8320	1.4387	2.3108	1.7196	0.0093
1.200	0.600	0.0782	6.3086	1.1253	1.8061	1.3298	0.0072
1.200	0.700	0.0663	4.5963	1.0277	1.6071	1.1469	0.0062
1.200	0.800	0.0634	3.8552	1.0760	1.6400	1.1125	0.0060
1.200	0.900	0.0704	3.3463	1.2105	1.7682	1.1877	0.0064
1.200	1.000	0.0829	3.0191	1.3301	1.8755	1.3024	0.0071
1.200	1.500	0.1383	1.5052	1.2869	1.5325	1.6286	0.0088
1.200	2.000	0.1643	0.9147	1.0862	1.1512	1.7450	0.0095
1.200	2.500	0.1796	0.6871	0.9317	0.9376	1.8156	0.0098
1.200	3.000	0.1835	0.7164	0.8296	0.9024	1.8413	0.0100
1.200	3.500	0.1794	0.8607	0.7494	0.9648	1.8293	0.0099
1.200	4.000	0.1747	0.9277	0.6628	0.9856	1.8051	0.0098
1.200	4.500	0.1728	0.8472	0.5933	0.9131	1.7844	0.0097
1.200	5.000	0.1731	0.6849	0.4945	0.7586	1.7680	0.0096
1.200	5.500	0.1740	0.5029	0.4720	0.5915	1.7608	0.0095
1.200	6.000	0.1738	0.3458	0.3544	0.4337	1.7502	0.0095
1.200	6.500	0.1730	0.2408	0.3102	0.3252	1.7484	0.0095
1.200	7.000	0.1713	0.1802	0.2690	0.2546	1.7466	0.0095
1.200	7.500	0.1691	0.1460	0.2846	0.2232	1.7449	0.0095
1.200	8.000	0.1670	0.1305	0.2914	0.2064	1.7431	0.0094
1.200	8.500	0.1650	0.1262	0.3618	0.2132	1.7466	0.0095
1.200	9.000	0.1632	0.1322	0.3643	0.2162	1.7466	0.0095
1.200	9.500	0.1615	0.1388	0.4601	0.2344	1.7484	0.0095
1.200	10.000	0.1604	0.1461	0.5464	0.2513	1.7518	0.0095
1.400	0.100	1.3296	H	0.6245	1.0919	1.7196	0.0093
1.400	0.200	0.3940	61.8242	1.0824	1.8748	2.4566	0.0133
1.400	0.300	0.2046	20.6081	1.4387	2.4236	2.4383	0.0132
1.400	0.400	0.1313	12.6172	1.4760	2.4448	2.0151	0.0109
1.400	0.500	0.0988	7.8857	1.3577	2.1744	1.6362	0.0089
1.400	0.600	0.0837	5.4674	1.2386	1.9370	1.4026	0.0076
1.400	0.700	0.0807	4.3372	1.1990	1.8297	1.3164	0.0071
1.400	0.800	0.0842	3.4847	1.1972	1.7697	1.3022	0.0071
1.400	0.900	0.0912	2.9602	1.2316	1.7556	1.3369	0.0072
1.400	1.000	0.1008	2.5641	1.2554	1.7315	1.3936	0.0076
1.400	1.500	0.1407	1.4140	1.2310	1.4575	1.6255	0.0088
1.400	2.000	0.1630	0.9813	1.0812	1.1841	1.7403	0.0094
1.400	2.500	0.1734	0.8327	0.9466	1.0325	1.7912	0.0097
1.400	3.000	0.1766	0.7969	0.8474	0.9645	1.8075	0.0098
1.400	3.500	0.1770	0.7796	0.7615	0.9175	1.8083	0.0098
1.400	4.000	0.1760	0.7433	0.6842	0.8645	1.7989	0.0098
1.400	4.500	0.1746	0.6787	0.6162	0.7944	1.7850	0.0097
1.400	5.000	0.1735	0.6022	0.5766	0.7251	1.7773	0.0096
1.400	5.500	0.1723	0.5297	0.5204	0.6487	1.7681	0.0096

(continued on the following page)

TABLE H-1. (continued)

Sigma g	MMAD	ET	TB	PU	TH	TOT	ER
1.400	6.000	0.1713	0.4534	0.4726	0.5696	1.7626	0.0096
1.400	6.500	0.1700	0.3836	0.4371	0.4981	1.7572	0.0095
1.400	7.000	0.1686	0.3337	0.4371	0.4530	1.7554	0.0095
1.400	7.500	0.1671	0.2804	0.4007	0.3909	1.7501	0.0095
1.400	8.000	0.1659	0.2456	0.4163	0.3599	1.7501	0.0095
1.400	8.500	0.1644	0.2220	0.4371	0.3390	1.7501	0.0095
1.400	9.000	0.1630	0.2062	0.4512	0.3228	1.7483	0.0095
1.400	9.500	0.1618	0.2019	0.4708	0.3182	1.7501	0.0095
1.400	10.000	0.1605	0.1971	0.5322	0.3223	1.7483	0.0095
1.600	0.100	1.0637	H	0.6144	1.0742	1.6755	0.0091
1.600	0.200	0.3431	30.9121	1.0332	1.7744	2.2848	0.0124
1.600	0.300	0.1859	15.7715	1.2915	2.1791	2.2014	0.0119
1.600	0.400	0.1262	9.0123	1.3577	2.1980	1.8621	0.0101
1.600	0.500	0.1040	6.5609	1.3211	2.0919	1.6288	0.0088
1.600	0.600	0.0964	4.8666	1.2932	1.9810	1.4980	0.0081
1.600	0.700	0.0973	3.7187	1.2562	1.8576	1.4390	0.0078
1.600	0.800	0.1011	3.0492	1.2466	1.7829	1.4272	0.0077
1.600	0.900	0.1081	2.6105	1.2333	1.7185	1.4504	0.0079
1.600	1.000	0.1149	2.1990	1.2468	1.6619	1.4806	0.0080
1.600	1.500	0.1441	1.3458	1.1704	1.3927	1.6340	0.0089
1.600	2.000	0.1607	1.0281	1.0519	1.1928	1.7234	0.0093
1.600	2.500	0.1690	0.8755	0.9500	1.0614	1.7662	0.0096
1.600	3.000	0.1726	0.7987	0.8529	0.9693	1.7826	0.0097
1.600	3.500	0.1740	0.7333	0.7838	0.8978	1.7885	0.0097
1.600	4.000	0.1736	0.6778	0.7286	0.8374	1.7850	0.0097
1.600	4.500	0.1729	0.6207	0.6712	0.7739	1.7785	0.0096
1.600	5.000	0.1720	0.5698	0.6411	0.7250	1.7761	0.0096
1.600	5.500	0.1709	0.5232	0.5971	0.6718	1.7702	0.0096
1.600	6.000	0.1697	0.4797	0.5749	0.6290	1.7682	0.0096
1.600	6.500	0.1684	0.4347	0.5564	0.5842	1.7627	0.0096
1.600	7.000	0.1671	0.4024	0.5335	0.5479	1.7590	0.0095
1.600	7.500	0.1660	0.3685	0.5380	0.5177	1.7590	0.0095
1.600	8.000	0.1649	0.3441	0.5208	0.4886	1.7554	0.0095
1.600	8.500	0.1635	0.3184	0.4996	0.4563	1.7501	0.0095
1.600	9.000	0.1623	0.2912	0.5198	0.4327	1.7466	0.0095
1.600	9.500	0.1615	0.2748	0.5299	0.4163	1.7466	0.0095
1.600	10.000	0.1605	0.2704	0.5246	0.4086	1.7448	0.0095
1.800	0.100	0.9670	61.8242	0.6014	1.0460	1.6255	0.0088
1.800	0.200	0.2995	31.5429	0.9714	1.6847	2.1303	0.0115
1.800	0.300	0.1699	12.6172	1.2148	2.0222	2.0311	0.0110
1.800	0.400	0.1265	8.0434	1.2924	2.0745	1.7987	0.0098
1.800	0.500	0.1118	5.5726	1.3021	2.0228	1.6442	0.0089
1.800	0.600	0.1089	4.0820	1.2846	1.9252	1.5605	0.0085
1.800	0.700	0.1099	3.2116	1.2562	1.8130	1.5108	0.0082
1.800	0.800	0.1145	2.7104	1.2420	1.7433	1.5125	0.0082
1.800	0.900	0.1201	2.3263	1.2399	1.6819	1.5297	0.0083
1.800	1.000	0.1249	1.9922	1.2224	1.6047	1.5401	0.0083

(continued on the following page)

TABLE H-1. (continued)

Sigma g	MMAD	ET	TB	PU	TH	TOT	ER
1.800	1.500	0.1468	1.3024	1.1323	1.3548	1.6454	0.0089
1.800	2.000	0.1594	1.0281	1.0435	1.1905	1.7134	0.0093
1.800	2.500	0.1660	0.8766	0.9538	1.0653	1.7468	0.0095
1.800	3.000	0.1694	0.7886	0.8893	0.9826	1.7670	0.0096
1.800	3.500	0.1707	0.7161	0.8107	0.8992	1.7696	0.0096
1.800	4.000	0.1709	0.6641	0.7714	0.8474	1.7744	0.0096
1.800	4.500	0.1707	0.6200	0.7178	0.7931	1.7736	0.0096
1.800	5.000	0.1700	0.5715	0.7034	0.7520	1.7730	0.0096
1.800	5.500	0.1692	0.5330	0.6557	0.7026	1.7689	0.0096
1.800	6.000	0.1682	0.4937	0.6467	0.6676	1.7668	0.0096
1.800	6.500	0.1670	0.4648	0.6263	0.6357	1.7611	0.0095
1.800	7.000	0.1660	0.4393	0.6020	0.6046	1.7592	0.0095
1.800	7.500	0.1652	0.4129	0.5933	0.5776	1.7573	0.0095
1.800	8.000	0.1640	0.3891	0.5828	0.5517	1.7537	0.0095
1.800	8.500	0.1632	0.3725	0.5952	0.5390	1.7537	0.0095
1.800	9.000	0.1624	0.3552	0.5693	0.5134	1.7501	0.0095
1.800	9.500	0.1615	0.3373	0.5828	0.4981	1.7484	0.0095
1.800	10.000	0.1607	0.3186	0.5828	0.4778	1.7449	0.0095
2.000	0.100	0.7598	61.8242	0.5920	1.0297	1.5784	0.0086
2.000	0.200	0.2664	21.0286	0.9240	1.5911	1.9877	0.0108
2.000	0.300	0.1632	10.7246	1.1486	1.9047	1.9289	0.0105
2.000	0.400	0.1307	6.5609	1.2258	1.9551	1.7619	0.0096
2.000	0.500	0.1201	4.4581	1.2668	1.9130	1.6507	0.0089
2.000	0.600	0.1198	3.4697	1.2764	1.8595	1.6105	0.0087
2.000	0.700	0.1209	2.8262	1.2442	1.7568	1.5707	0.0085
2.000	0.800	0.1250	2.4393	1.2256	1.6924	1.5741	0.0085
2.000	0.900	0.1286	2.1269	1.2240	1.6351	1.5807	0.0086
2.000	1.000	0.1324	1.8926	1.2021	1.5712	1.5890	0.0086
2.000	1.500	0.1489	1.2821	1.1167	1.3387	1.6596	0.0090
2.000	2.000	0.1590	1.0222	1.0286	1.1798	1.7105	0.0093
2.000	2.500	0.1639	0.8874	0.9647	1.0780	1.7379	0.0094
2.000	3.000	0.1668	0.7823	0.8979	0.9832	1.7506	0.0095
2.000	3.500	0.1681	0.7210	0.8493	0.9214	1.7601	0.0095
2.000	4.000	0.1686	0.6659	0.8029	0.8632	1.7653	0.0096
2.000	4.500	0.1685	0.6194	0.7686	0.8153	1.7666	0.0096
2.000	5.000	0.1681	0.5797	0.7406	0.7738	1.7660	0.0096
2.000	5.500	0.1675	0.5456	0.7083	0.7340	1.7638	0.0096
2.000	6.000	0.1668	0.5162	0.6946	0.7049	1.7635	0.0096
2.000	6.500	0.1660	0.4817	0.6943	0.6758	1.7596	0.0095
2.000	7.000	0.1652	0.4673	0.6661	0.6524	1.7595	0.0095
2.000	7.500	0.1644	0.4363	0.6520	0.6196	1.7540	0.0095
2.000	8.000	0.1637	0.4166	0.6358	0.5955	1.7521	0.0095
2.000	8.500	0.1630	0.4004	0.6514	0.5848	1.7520	0.0095
2.000	9.000	0.1623	0.3920	0.6325	0.5695	1.7502	0.0095
2.000	9.500	0.1616	0.3748	0.6358	0.5530	1.7484	0.0095
2.000	10.000	0.1611	0.3659	0.6245	0.5402	1.7466	0.0095

(continued on the following page)

TABLE H-1. (continued)

Sigma g	MMAD	ET	TB	PU	TH	TOT	ER
2.200	0.100	0.6648	61.8242	0.5859	1.0191	1.5485	0.0084
2.200	0.200	0.2416	15.7715	0.8946	1.5204	1.8827	0.0102
2.200	0.300	0.1593	9.1925	1.0748	1.7733	1.8340	0.0099
2.200	0.400	0.1354	5.4674	1.1714	1.8342	1.7305	0.0094
2.200	0.500	0.1281	4.0078	1.2171	1.8312	1.6684	0.0090
2.200	0.600	0.1279	3.1543	1.2223	1.7711	1.6331	0.0089
2.200	0.700	0.1289	2.6636	1.2157	1.7168	1.6155	0.0088
2.200	0.800	0.1319	2.2869	1.2093	1.6555	1.6141	0.0087
2.200	0.900	0.1351	2.0119	1.2031	1.6000	1.6202	0.0088
2.200	1.000	0.1382	1.8025	1.1814	1.5382	1.6251	0.0088
2.200	1.500	0.1510	1.2417	1.1004	1.3147	1.6710	0.0091
2.200	2.000	0.1582	1.0029	1.0339	1.1742	1.7052	0.0092
2.200	2.500	0.1625	0.8746	0.9767	1.0775	1.7310	0.0094
2.200	3.000	0.1649	0.7886	0.9238	1.0011	1.7444	0.0095
2.200	3.500	0.1661	0.7121	0.8656	0.9234	1.7482	0.0095
2.200	4.000	0.1666	0.6673	0.8283	0.8760	1.7538	0.0095
2.200	4.500	0.1667	0.6249	0.8055	0.8357	1.7593	0.0095
2.200	5.000	0.1664	0.5951	0.7702	0.7982	1.7589	0.0095
2.200	5.500	0.1660	0.5542	0.7622	0.7633	1.7585	0.0095
2.200	6.000	0.1655	0.5306	0.7325	0.7320	1.7564	0.0095
2.200	6.500	0.1649	0.5118	0.7349	0.7172	1.7581	0.0095
2.200	7.000	0.1644	0.4927	0.7263	0.6973	1.7579	0.0095
2.200	7.500	0.1637	0.4686	0.7166	0.6721	1.7541	0.0095
2.200	8.000	0.1633	0.4489	0.6934	0.6457	1.7522	0.0095
2.200	8.500	0.1627	0.4287	0.6800	0.6218	1.7504	0.0095
2.200	9.000	0.1622	0.4206	0.6857	0.6153	1.7503	0.0095
2.200	9.500	0.1615	0.4122	0.6780	0.6043	1.7467	0.0095
2.200	10.000	0.1610	0.3911	0.6842	0.5851	1.7450	0.0095
2.400	0.100	0.5674	63.0859	0.5769	1.0125	1.5217	0.0082
2.400	0.200	0.2278	15.7715	0.8548	1.4548	1.8103	0.0098
2.400	0.300	0.1595	7.1497	1.0339	1.6730	1.7783	0.0096
2.400	0.400	0.1404	4.7765	1.1276	1.7577	1.7420	0.0093
2.400	0.500	0.1350	3.5859	1.1823	1.7573	1.6838	0.0091
2.400	0.600	0.1344	2.9440	1.1990	1.7297	1.6637	0.0090
2.400	0.700	0.1357	2.4799	1.2042	1.6787	1.6524	0.0090
2.400	0.800	0.1377	2.1523	1.1819	1.6082	1.6430	0.0089
2.400	0.900	0.1398	1.8764	1.1710	1.5430	1.6385	0.0089
2.400	1.000	0.1424	1.6918	1.1604	1.4942	1.6451	0.0089
2.400	1.500	0.1523	1.2217	1.0832	1.2975	1.6795	0.0091
2.400	2.000	0.1579	1.0127	1.0239	1.1746	1.7074	0.0093
2.400	2.500	0.1614	0.8803	0.9714	1.0783	1.7240	0.0093
2.400	3.000	0.1637	0.7954	0.9337	1.0102	1.7401	0.0094
2.400	3.500	0.1645	0.7284	0.8921	0.9476	1.7442	0.0095
2.400	4.000	0.1651	0.6687	0.8649	0.8942	1.7480	0.0095
2.400	4.500	0.1651	0.6309	0.8345	0.8533	1.7497	0.0095
2.400	4.500	0.1651	0.6002	0.8216	0.8248	1.7534	0.0095
2.400	5.500	0.1648	0.5757	0.8070	0.7994	1.7550	0.0095

(continued on the following page)

TABLE H-1. (continued)

Sigma g	MMAD	ET	TB	PU	TH	TOT	ER
2.400	6.000	0.1645	0.5512	0.7798	0.7682	1.7548	0.0095
2.400	6.500	0.1641	0.5267	0.7744	0.7457	1.7546	0.0095
2.400	7.000	0.1636	0.5022	0.7568	0.7178	1.7508	0.0095
2.400	7.500	0.1633	0.4900	0.7494	0.7036	1.7525	0.0095
2.400	8.000	0.1629	0.4701	0.7286	0.6779	1.7506	0.0095
2.400	8.500	0.1625	0.4622	0.7362	0.6730	1.7524	0.0095
2.400	9.000	0.1619	0.4497	0.7312	0.6593	1.7487	0.0095
2.400	9.500	0.1616	0.4290	0.7061	0.6308	1.7468	0.0095
2.400	10.000	0.1611	0.4206	0.6994	0.6201	1.7450	0.0095

*H = Humans receive some deposition, but rats do not.

R = Rats receive some deposition, but humans do not.

Source: adapted from Jarabek et al., 1989a.

The ratio is the extrarespiratory RDDs calculated for the experimental species and human then is used to calculate the HEC Equation 4-7:

$$\mathrm{NOAEL}_{[HEC]}(\mathrm{mg/m^3}) = \mathrm{NOAEL}_{[ADJ]}(\mathrm{mg/m^3}) \times \mathrm{RDDR}_{ER}$$

where:

$\mathrm{NOAEL}_{[HEC]}$ = the NOAEL human equivalent concentration,

$\mathrm{NOAEL}_{[ADJ]}$ = the NOAEL adjusted for duration according to Equation 4-3, and

$\mathrm{RDDR}_{ER} = (\mathrm{RDD}_{ER})_A/(\mathrm{RDD}_{ER})_H$, the ratio of the dose available for the entire respiratory system of the experimental animal species to that of humans.

It should be noted that body weight and not surface area is in the denominator of the calculation for RDD for ER effects. THE RDDR VALUES IN TABLE H-1 FOR ER EFFECTS DO NOT HAVE BODY WEIGHT FACTORED IN AT THIS TIME, PENDING RESOLUTION ON RECOMMENDED VALUES FOR BODY WEIGHTS, (SEE SECTION 4.1.1.4). THUS, THESE RATIOS WILL NEED TO BE MULTIPLIED BY $(BW)_H/(BW)_A$ WHEN USED. THOSE VALUES FOR WHICH AN "H" APPEARS INDICATE NUMBERS FOR WHICH HUMANS RECEIVE SOME DEPOSITION BUT RATS DO NOT. THE "R"s INDICATE VALUES FOR WHICH RATS RECEIVE SOME DEPOSITION AND HUMANS DO NOT. IN THESE CASES, RDD VALUES MAY PROVIDE SOME INSIGHT ON THE ASSESSMENT, BUT SHOULD BE DISCUSSED WITH AN EPA SCIENTIST FIRST.

A plot of the RDDR for rats vs. humans for the TB region is shown in Figure H-2 and for the PU region in Figure H-3. The plots show two different standard deviations of aerosol distributions, a σ_g of 1.4 and 2.4 (essentially monodisperse and polydisperse distributions), to illustrate the sensitivity of the burden ratios to that parameter. The line is drawn across the plot from the RDDR value of 1.0 as a demarcation. Values of RDDR greater than 1.0 indicate where the rat receives more of an inhaled dose relative to humans, and thus adjustment by the RDDR would result in a larger $NOAEL_{HEC}$ than the animal $NOAEL_{ADJ}$ estimate. Below the demarcation line, the animals receive less of that characteristic dose relative to humans, and adjustment by the RDDR would result in a decreased $NOAEL_{HEC}$ relative to the animal $NOAEL_{ADJ}$ estimate. Note that the rat receives a much higher burden in the TB region (Figure H-2) relative to humans for particles less than 2 µm, while humans receive higher relative doses in the TB region for particles greater than 2 µm. With the exception of the particle size range of 0.2 to 2 µm, where the rat receives more, humans receive a greater dose relative to rats across the entire particle size range in the PU region (Figure H-3), and the equivalent exposure concentrations would be scaled downward. These plots help to illustrate the effect of dosimetric adjustment on the apparent (observed) effect concentration.

The influence of breathing route (i.e., nose-breathing with normal augmentation through the mouth vs. mouth breathing alone) on DDRs is significant as illustrated in Figure H-4, plots A vs. B. The total RDDR for mouth breathers (B) is higher for the entire particle size range in comparison to normal augmenters (A). This difference emphasizes the need for an activity pattern scenario for humans (e.g., x hours rest, y hours light activity, z hours heavy exercise) to account for changes in deposition pattern due to breathing patterns, rather than calculating RDDRs for humans using an assumed default ventilatory parameter (i.e., 20 m^3/day or 13.8 ℓ/min). A range in minute ventilation from 12 to 132 ℓ/min has been associated with representative types of exercise from light to severe (U.S. Environmental Protection Agency, 1986c). Humans normally augment respiratory airflow with oronasal breathing when minute ventilation exceeds approximately 35 ℓ/min (U.S. Environmental Protection Agency, 1986c), and this breathing mode significantly alters the regional deposition of inhaled particles (Miller et al., 1988). This alteration in regional deposition then significantly alters the RDDR used to adjust the experimental exposure concentration to a human equivalent concentration,

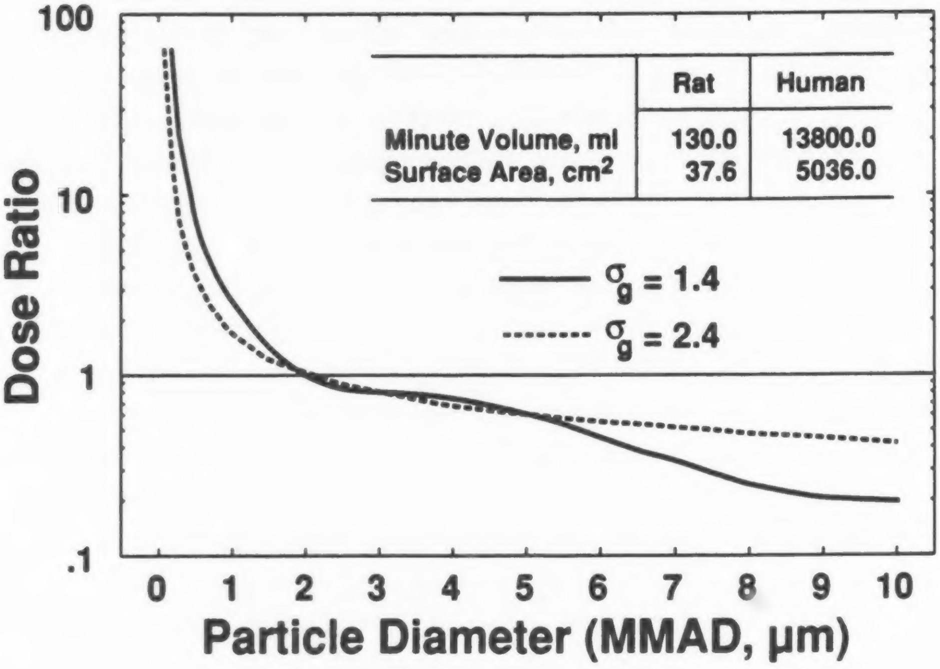


Figure H-2. RDDR of the rat to the human by particle diameter (MMAD) for the TB region.

Source: Jarabek et al., 1989a.

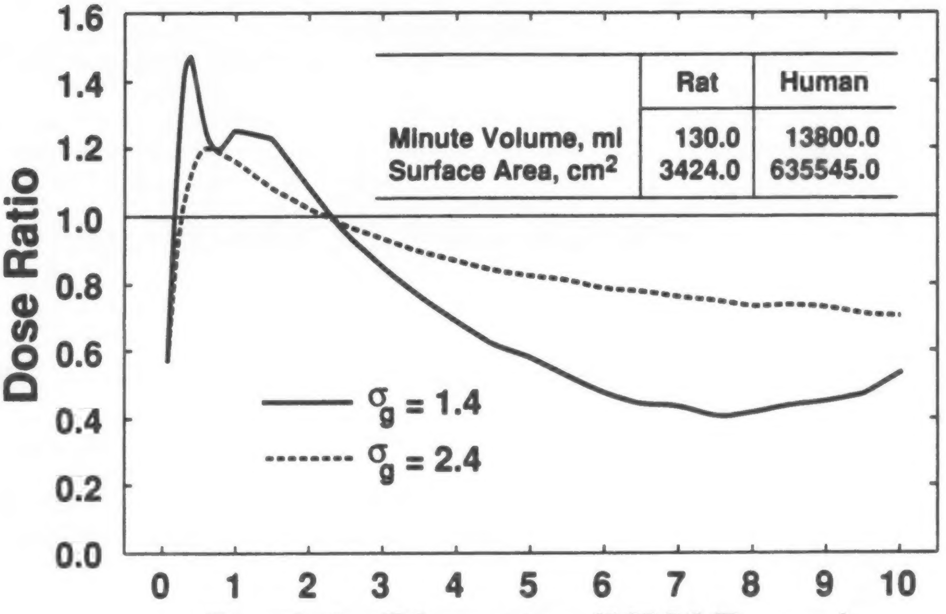


Figure H-3. RDDR of the rat to the human by particle diameter (MMAD) for the PU region.

Source: Jarabek et al. 1989a.

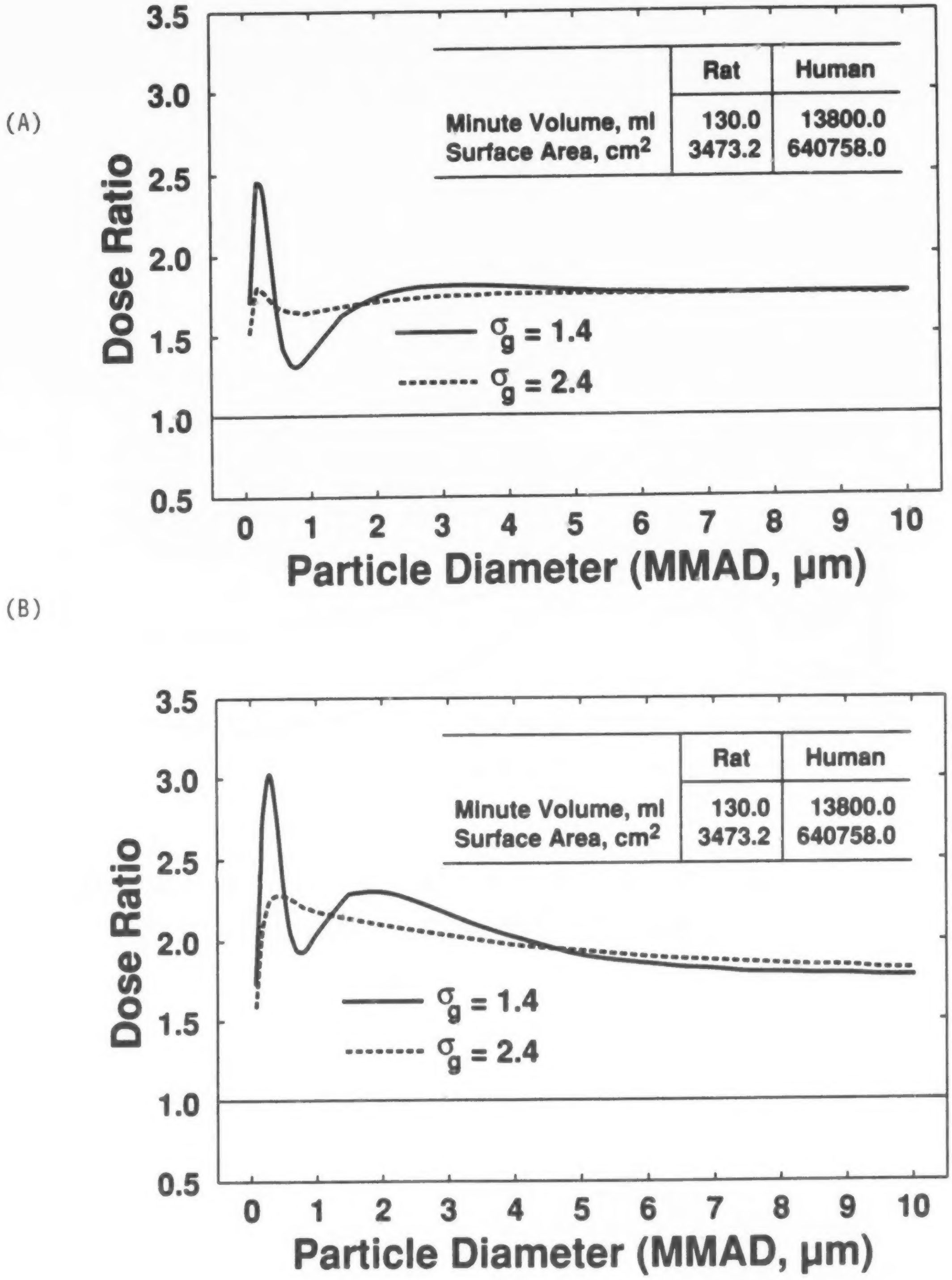


Figure H-4. RDDR of the rat to the human by particle diameter (MMAD) for the TOT system in (A) normal augmenters and (B) mouth breathers. A proportionality factor for the split in air flow between nose and mouth (Niinimaa et al., 1981) was used in human deposition calculation for plot (A).

Source: Jarabek et al. 1989a.

and thus, significantly alters the derived RfD_i. Computation of a representative activity pattern for humans as proposed will make better use of models that estimate deposition burdens as a function of the complex interaction between breathing route, ventilation level, and particle aerodynamic properties. This will provide a more realistic estimate of probable human exposure.

EXAMPLE CALCULATIONS

Ep(a)oxide is a hypothetical noxious agent found as a insoluble particulate emission from municipal waste combustion sources, and there is a need to calculate a proposed RfD_i. Associated health effects of ep(a)oxide include both central nervous system (CNS) and respiratory functional and structural abnormalities. Recently, two well-conducted, chronic inhalation toxicology investigations have been performed by two different laboratories that evaluate these effects in rats. The NOAELS of the critical effect data evaluated in these investigations are summarized in Table H-2, but since dosimetric adjustments have not been made for the exposure conditions or the observed toxic effects, comparison is not possible. The following outlines the steps which would need to be executed to perform this adjustment. It should be noted that in this example both investigations were performed on the rat, while other studies may require that an RDDR be tabulated for other species in question.

Equation 4-3 would first be applied to the results in order to adjust for the discontinuous exposure protocol.

$$\mathrm{NOAEL}_{[\mathrm{ADJ}]}\ (\mathrm{mg/m^3}) = \mathrm{E}\ (\mathrm{mg/m^3}) \times \mathrm{D}\ (\mathrm{hours/day/24/hours}) \times \mathrm{W}\ (\mathrm{days/7\ days})$$

where: E = experimental exposure level,

D = number of hours exposed/day/24 hours, and

W = number of days of exposure/7 days.

The calculation for duration adjustment of the Laboratory 1 exposure is:

$$\begin{aligned}\mathrm{NOAEL}_{[\mathrm{ADJ}]}(\mathrm{mg/m^3}) &= 120.0 \times 8/24 \times 5/7 \\ &= 29\ \mathrm{mg/m^3}.\end{aligned}$$

The calculation for ep(a)oxide results from Laboratory 2 is given by:

$$\begin{aligned}\mathrm{NOAEL}_{[\mathrm{ADJ}]}\ (\mathrm{mg/m^3}) &= 12 \times 8/24 \times 5/7 \\ &= 2.9\ \mathrm{mg/m^3}.\end{aligned}$$

TABLE H-2. SUMMARY OF SYSTEMIC TOXICITY NOAELS* FOR EP(a)OXIDE OBSERVED IN FISCHER 344 RATS

Exposure	Duration	System Examined	Effects	Reference
120 mg/m³ MMAD = 2.0 μm σ_g = 1.6	8 h/day 5 days/week for 9 months	CNS	No exposure-related effects on EMG or limb tremor	Lab 1
12 mg/m³ MMAD = 0.2 μm σ_g = 1.8	8 h/day 5 days/week for 12 months	Respiratory	No exposure-related decrease in mucociliary clearance or alterations in epithelial architecture/goblet cell hypertrophy	Lab 2

*It Should be noted that only the NOAEL data (adverse effects occurred at higher exposure concentrations in each investigation) is provided for this ep(a)oxide and not a full data array. Choice of toxicity data is discussed in Appendix G and entails an analysis of all data, NOAEL/LOAEL interfaces, and such. This table is provided only to illustrate the dosimetric adjustments.

The RDDR for each exposure condition and toxicologic effect then is calculated by using Table H-2.

The effect of interest is an ER effect for the exposure conditions (σ_g = 1.6, MMAD = 2.0 μm) investigated by Laboratory 1 so that an RDDR corresponding to a σ_g of 1.6 and MMAD of 2.0 should be read from the ER column (see page H-7). The resulting RDDR is 0.0093. However, as previously discussed, these values in Table H-1 for $RDDR_{ER}$ do not have the ratio of body weights factored in, so this value will need to be adjusted by $(BW)_H/(BW)_A$. The default value for body weight for male Fischer 344 rats is .38 kg (U.S. Environmental Protection Agency, 1988c), and the default body weight for humans is 70 kg, thus, .0093 multiplied by 70/.38 results in a $RDDR_{ER}$ of 1.7. This ratio then is used in Equation 4-7 to calculate the $NOAEL_{HEC}$ for ER effects as:

$$\begin{aligned} NOAEL_{[HEC]}(mg/m^3) &= NOAEL_{[ADJ]}(mg/m^3) \times RDDR_{ER}. \\ &= 29 \times 1.7 \\ &= 49.3\ mg/m^3 \end{aligned}$$

For the results of Laboratory 2, an RDDR is calculated for only the TB region since measurements of mucociliary clearance and histopathology were used to assess effects in the tracheobronchial region. Therefore, dose adjustment by the TB region RDDR value is appropriate. The RDDR for the TB region corresponding to an exposure condition of σ_g = 1.8 and an MMAD = 0.2 µm is 31.54 (see page H-7).

Equation 4-5 then is used to adjust the exposure effect levels for dosimetric differences as follows:

$$\mathrm{NOAEL}_{[\mathrm{HEC}]}\ (\mathrm{mg/m^3}) = \mathrm{NOAEL}_{[\mathrm{ADJ}]}\ (\mathrm{mg/m^3}) \times \mathrm{RDDR}_{\mathrm{PU}}.$$

The NOAEL observed in the investigations of Laboratory 2 adjusted for dosimetric differences is:

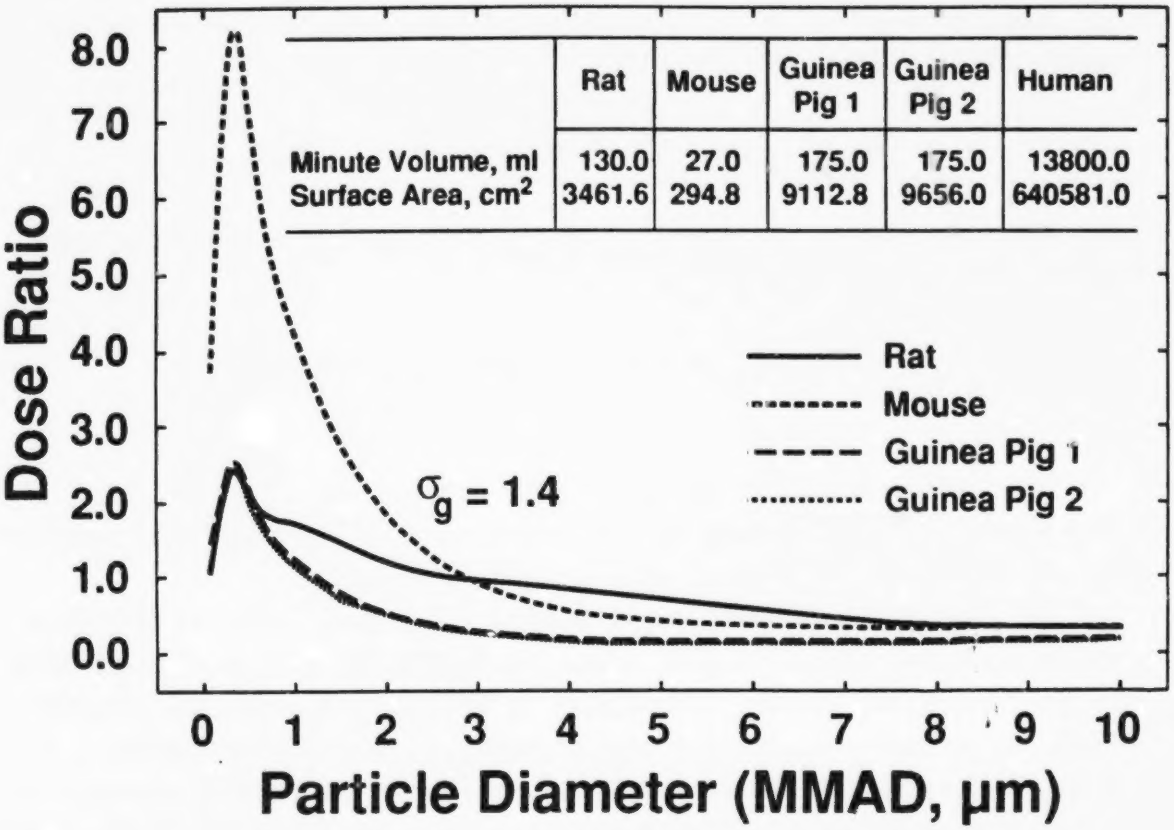
$$\begin{aligned}\mathrm{NOAEL}_{[\mathrm{HEC}]}\ (\mathrm{mg/m^3}) &= 2.9\ \mathrm{mg/m^3}_{[\mathrm{ADJ}]} \times 31.54.\\ &= 91.5\ \mathrm{mg/m^3}\end{aligned}$$

Thus, after dosimetric adjustment, the $\mathrm{NOAEL}_{\mathrm{HEC}}$ for ER effects (CNS) of 49.3 $\mathrm{mg/m^3}$ from the investigations of Laboratory 1 is lower than that observed for the TB effects (91.5 $\mathrm{mg/m^3}$) observed by Laboratory 2.

This emphasizes the need for dosimetric adjustments prior to data array analysis and key study selection, since, as in this example, an observed NOAEL in the same animal species that appears to be 10-fold greater than another NOAEL may actually result in a smaller $\mathrm{NOAEL}_{\mathrm{HEC}}$ once such adjustments are made. Dosimetric adjustments also will affect comparisons across species. As illustrated in Figure H-5, exposure to rats, mice, and guinea pigs to the same exposure concentration with an MMAD of 2.0 µm and a σ_g of 1.4 would result in different $\mathrm{NOAEL}_{\mathrm{HEC}}$ estimates (1.1, 1.7 and .74 times the exposure concentration, respectively). Again, this illustration emphasizes the need to correct exposure concentrations to human equivalents before choosing the critical effect and key study.

RESEARCH AND DEVELOPMENT

The EPA recognizes that the establishment of RfD_is critically depends on the quantitative extrapolation of regional respiratory tract doses from animals to humans. The RDDR as described in this Appendix must address both the



	Rat	Mouse	Guinea Pig 1	Guinea Pig 2	Human
Minute Volume, ml	130.0	27.0	175.0	175.0	13800.0
Surface Area, cm^2	3461.6	294.8	9112.8	9656.0	640581.0

Figure H-5. RDDR of three species to the human by particle diameter (MMAD) for the TH region. Guinea pig 1 and 2 refer to calculations using different lung surface area data.

Source: Jarabek et al. 1989b.

deposition and fate of deposited particles to adequately accomplish this. That is, factors must be incorporated into the RDDR derivation which account for the continuous redistribution and clearance of inhaled particles within the lungs of the species of interest to risk assessment (including humans) during chronic exposures.

A work group has been formed with members of ECAO-RTP and HERL to expand the current RDDR methodology, using empirical data and existing theoretical models to incorporate clearance and to derive a similar dose adjustment factor for gas and vapor exposures. The mission of this group is to incorporate into the methodology for particles as many of the following factors as is feasible.

- Regional Deposition
  - particle size
  - particle distribution (σ_g)
  - particle volatility or hygroscopicity
  - detailed regional respiratory tract morphology for multiple species
  - extrathoracic and intrathoracic deposition
  - alternative modes of breathing (nasal, oronasal, and oral) and activity patterns

- Fate of Inhaled Particles
  - mucociliary transport and clearance
  - alveolar clearance
  - phagocytosis and translocation by macrophages
  - dissolution
  - free particle translocation
  - particle solubility
  - chemical activity: local vs. systemic

Pepelko (1987) investigated the feasibility of dose adjustments based on reported pulmonary clearance rates. The bioavailabilities of single inhaled doses of particulate matter having dissolution half-times ranging from one day to over five years were estimated by calculating the amount dissolved each day and summing over a two-year period. Two years was selected because it approximated the remaining lifetime of an exposed rat.

The equation used to carry out this calculation is:

$$\text{Total bioavailable percentage} = \frac{100k_s}{k_p + k_s} \quad 1-e^{-(k_p + k_s)t}$$

where:

k_p = the rate constant for elimination via physical transport of particles from the lungs

k_s = the rate constant for particle dissolution

and

t = time in days.

Values of 60 and 240 days were selected as representative of physical clearance rates in rats and humans, respectively. It should be cautioned that these values were selected only as examples, since actual clearance rates are somewhat uncertain and vary with conditions.

The results are shown in Figure H-6. As can be seen, for very short dissolution half-time ($t_{1/2s}$) values, physical clearance rates had little effect upon total bioavailability. In fact, for a $t_{1/2s}$ of one day, the calculated bioavailable percentages were 98.4 and 99.6 for particle removal half-time ($t_{1/2p}$) values of 60 and 240 days, respectively. On the other hand, when $t_{1/2s}$ is increased to 120 days, the estimated bioavailability equals only 32% for a $t_{1/2p}$ of 60 days, compared to 67% when the $t_{1/2p}$ is equal to 240 days. For particles with very long dissolution half-times, the total bioavailability is predicted to be small in both cases, although the relative amount will continue to be up to three times as great when the $t_{1/2p}$ equals 240 days.

Other uncertainties in the estimation of bioavailability result from regional and interspecies differences in physiology. Particles deposited in alveolar regions, for example, are almost invariably taken up by phagocytic cells, which have been shown to alter the rate of dissolution (Andre et al., 1987). Considerable quantities of particles are transported to and stored in the lung-associated lymph nodes of dogs (Snipes et al., 1983). Since this material is still in the body and subject to dissolution and absorption, use of reported clearance half-times will result in an underestimate of bioavailability, unless the rates of translocation to the lymph nodes are known, allowing an appropriate adjustment to be made. Certain metals, such as

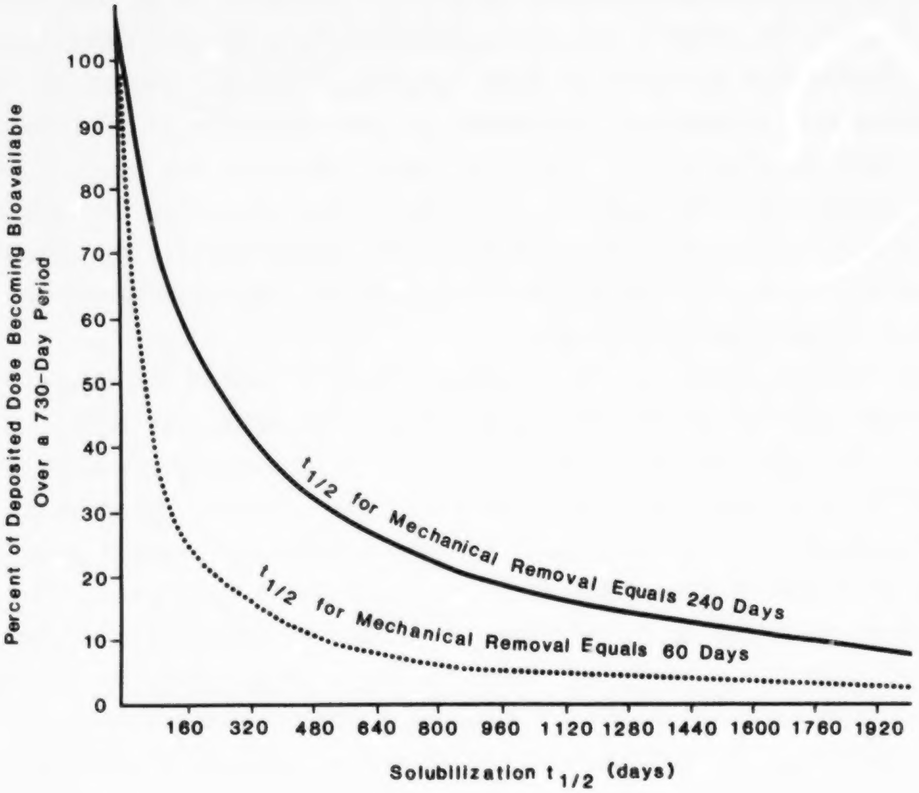


Figure H-6. The relationship between particle removal half-time ($t_{1/2p}$) and dissolution half-time ($t_{1/2s}$) upon the bioavailability of a single deposited dose of inhaled particulate matter over a 730-day period.

Source: Pepelko (1987).

beryllium, cadmium, lead, and arsenic have very long-term clearance components (Rhoads and Sanders, 1985; Reeves et al., 1967). While the slow clearance may be partially ascribed to toxicity, at least a portion was considered by the authors to be due to uptake by lung cells and formation of a stable complex with metallothionein-like proteins. Although there also is some evidence that alveolar clearance is better described by two exponential rate constants than one, in both small animals (Snipes et al., 1983) and in humans (Bohning et al., 1982), only a single value has been reported in most studies.

The use of reported clearance rates also may result in an underestimate of bioavailability in animals when extrapolating from a chronic toxicology study, because continuous exposure at high concentrations may result in lung overloading with concomitant decreases, or even cessation of clearance (Chan et al., 1984; Griffis et al., 1983). Further, there are few comparisons across species using the same type of particles. This investigation helps to illustrate the interaction of clearance with bioavailability for chronic dose adjustments and serves to emphasize that these and other considerations must be addressed in the model development.

The initial output of the research effort to expand the scope of the methodology will be an analytic model from which RRDRs for particles are derived. The most difficult task of the research work group will be the development of a model that satisfies all of the criteria listed on page H-19. The achievement of this goal will involve compromises between scientific accuracy and general applicability in risk assessment procedures. The project has already identified some data gaps that has initiated an investigation to obtain regional surface area and clearance rates using consistent methodologies across species in order to ensure compatible and precise estimates for model input. The output is anticipated to be a support document of RRDR tables to be used in the RfD_i risk assessment methodology for dose adjustment and reduction of uncertainty in interspecies extrapolation for aerosol exposures. Specifications on how to apply these ratios as scaling factors and limitations (e.g., duration of exposures) will be explicitly stated. Compilation of regional surface area data, using consistent inflation, fixation, and morphometry techniques across species, will facilitate investigation of the limitations on linear extrapolation of minute volumes and surface areas as well as the allometric relationships between lung weight, lung surface area and body weight. Further, it is expected that the characterization of anatomic and physiologic parameters across species, involved in the development of the aerosol model, will provide the basis for mass transport estimates needed to expand and refine existing gas deposition and uptake models (e.g., ozone and volatile organics). A gas and vapor model which accounts simultaneously for characteristics such as solubility, reactivity, and metabolic transformation then may be developed (see Appendix I). A similar support document of adjustment factors for these agents is envisioned.

OLD HOLD

TABLE H-1. RDDR VALUES BY MASS MEDIAN DIAMETER AND STANDARD DEVIATION FOR RATS*

Sigma g	MMAD	ET	TB	PU	Total
1.40	0.20	0.6	109.8	0.9	1.6
1.40	0.50	0.2	10.4	0.9	1.6
1.40	1.00	0.2	2.7	1.0	1.9
1.40	1.50	0.4	1.4	0.9	2.6
1.40	2.00	0.5	1.0	0.8	3.1
1.40	2.50	0.7	0.8	0.8	3.6
1.40	3.00	0.9	0.8	0.7	4.0
1.40	3.50	1.2	0.8	0.6	4.4
1.40	4.00	1.5	0.7	0.6	4.9
1.40	4.50	1.9	0.7	0.5	5.3
1.40	5.00	2.4	0.6	0.5	5.9
1.40	5.50	2.9	0.5	0.5	6.4
1.40	6.00	3.5	0.5	0.4	7.0
1.40	6.50	4.2	0.4	0.4	7.7
1.40	7.00	4.9	0.3	0.4	8.3
1.40	7.50	5.8	0.3	0.4	9.1
1.40	8.00	6.3	0.3	0.4	9.9
1.40	8.50	7.0	0.2	0.4	10.6
1.40	9.00	7.8	0.2	0.4	11.5
1.40	9.50	8.8	0.2	0.5	12.4
1.40	10.00	9.4	0.2	0.5	13.4
1.60	0.20	0.5	54.9	0.8	1.6
1.60	0.50	0.2	8.5	0.9	1.6
1.60	1.00	0.3	2.3	0.9	2.1
1.60	1.50	0.4	1.4	0.9	2.7
1.60	2.00	0.6	1.0	0.8	3.2
1.60	2.50	0.7	0.9	0.8	3.6
1.60	3.00	0.9	0.8	0.7	4.0
1.60	3.50	1.1	0.7	0.6	4.5
1.60	4.00	1.4	0.7	0.6	4.9
1.60	4.50	1.7	0.6	0.6	5.4
1.60	5.00	2.1	0.6	0.6	5.9
1.60	5.50	2.5	0.5	0.5	6.4
1.60	6.00	2.9	0.5	0.5	6.9
1.60	6.50	3.4	0.4	0.5	7.5
1.60	7.00	4.0	0.4	0.5	8.1
1.60	7.50	4.5	0.4	0.5	8.8
1.60	8.00	5.1	0.4	0.5	9.4
1.60	8.50	5.8	0.3	0.5	10.2
1.60	9.00	6.3	0.3	0.5	10.8
1.60	9.50	7.0	0.3	0.5	11.6
1.60	10.00	7.7	0.3	0.5	12.4

(continued on the following page)

TABLE H-1. (continued)

Sigma g	MMAD	ET	TB	PU	Total
1.80	0.20	0.4	54.9	0.8	1.6
1.80	0.50	0.2	7.3	0.9	1.7
1.80	1.00	0.3	2.2	0.9	2.2
1.80	1.50	0.4	1.3	0.9	2.8
1.80	2.00	0.6	1.0	0.8	3.2
1.80	2.50	0.8	0.9	0.8	3.7
1.80	3.00	0.9	0.8	0.7	4.1
1.80	3.50	1.1	0.7	0.7	4.6
1.80	4.00	1.4	0.7	0.6	5.0
1.80	4.50	1.6	0.6	0.6	5.4
1.80	5.00	1.9	0.6	0.6	5.9
1.80	5.50	2.2	0.5	0.6	6.4
1.80	6.00	2.6	0.5	0.5	6.9
1.80	6.50	2.9	0.5	0.5	7.4
1.80	7.00	3.3	0.4	0.5	8.0
1.80	7.50	3.7	0.4	0.5	8.5
1.80	8.00	4.2	0.4	0.5	9.1
1.80	8.50	4.7	0.4	0.5	9.8
1.80	9.00	5.3	0.4	0.5	10.4
1.80	9.50	5.7	0.4	0.5	11.0
1.80	10.00	6.3	0.4	0.5	11.8
2.00	0.20	0.4	36.6	0.8	1.5
2.00	0.50	0.2	5.9	0.9	1.7
2.00	1.00	0.4	2.1	0.9	2.3
2.00	1.50	0.5	1.3	0.9	2.9
2.00	2.00	0.6	1.0	0.8	3.3
2.00	2.50	0.8	0.9	0.8	3.8
2.00	3.00	1.0	0.8	0.7	4.2
2.00	3.50	1.1	0.7	0.7	4.6
2.00	4.00	1.4	0.7	0.7	5.0
2.00	4.50	1.6	0.6	0.6	5.5
2.00	5.00	1.8	0.6	0.6	5.9
2.00	5.50	2.1	0.5	0.6	6.4
2.00	6.00	2.4	0.5	0.6	6.9
2.00	6.50	2.6	0.5	0.6	7.3
2.00	7.00	3.0	0.5	0.6	7.8
2.00	7.50	3.3	0.5	0.6	8.3
2.00	8.00	3.7	0.5	0.6	9.0
2.00	8.50	4.1	0.4	0.6	9.5
2.00	9.00	4.5	0.4	0.6	10.1
2.00	9.50	4.9	0.4	0.5	10.6
2.00	10.00	5.3	0.4	0.6	11.3

(continued on the following page)

TABLE H-1. (continued)

Sigma g	MMAD	ET	TB	PU	Total
2.20	0.20	0.3	27.4	0.8	1.5
2.20	0.50	0.3	5.3	0.9	1.8
2.20	1.00	0.4	2.0	0.9	2.4
2.20	1.50	0.5	1.3	0.9	2.9
2.20	2.00	0.7	1.0	0.8	3.4
2.20	2.50	0.8	0.9	0.8	3.9
2.20	3.00	1.0	0.8	0.7	4.3
2.20	3.50	1.2	0.7	0.7	4.7
2.20	4.00	1.3	0.7	0.7	5.1
2.20	4.50	1.5	0.6	0.7	5.5
2.20	5.00	1.7	0.6	0.7	5.9
2.20	5.50	2.0	0.6	0.6	6.4
2.20	6.00	2.2	0.6	0.6	6.8
2.20	6.50	2.5	0.5	0.6	7.3
2.20	7.00	2.7	0.5	0.6	7.8
2.20	7.50	3.0	0.5	0.6	8.3
2.20	8.00	3.3	0.5	0.6	8.7
2.20	8.50	3.6	0.5	0.6	9.2
2.20	9.00	3.9	0.5	0.6	9.8
2.20	9.50	4.3	0.5	0.6	10.3
2.20	10.00	4.7	0.5	0.6	10.9
2.40	0.20	0.3	27.8	0.8	1.5
2.40	0.50	0.3	4.8	0.9	1.9
2.40	1.00	0.4	1.9	0.9	2.5
2.40	1.50	0.6	1.3	0.9	3.0
2.40	2.00	0.7	1.0	0.8	3.5
2.40	2.50	0.9	0.9	0.8	3.9
2.40	3.00	1.0	0.8	0.8	4.4
2.40	3.50	1.2	0.7	0.7	4.7
2.40	4.00	1.3	0.7	0.7	5.2
2.40	4.50	1.5	0.7	0.7	5.6
2.40	5.00	1.7	0.6	0.7	6.0
2.40	5.50	1.9	0.6	0.7	6.4
2.40	6.00	2.1	0.6	0.7	6.8
2.40	6.50	2.3	0.6	0.7	7.2
2.40	7.00	2.5	0.5	0.7	7.7
2.40	7.50	2.8	0.5	0.6	8.2
2.40	8.00	3.0	0.5	0.6	8.6
2.40	8.50	3.3	0.5	0.6	9.0
2.40	9.00	3.6	0.5	0.6	9.5
2.40	9.50	3.9	0.5	0.6	10.1
2.40	10.00	4.2	0.5	0.6	10.5

*Source: Jarabek et al., 1988.

APPENDIX I
DERIVATION OF AN APPROACH TO DETERMINE HUMAN EQUIVALENT CONCENTRATIONS FOR EXTRARESPIRATORY EFFECTS OF GAS EXPOSURES BASED ON A PB-PK MODEL USING SELECTED PARAMETER VALUES

INTRODUCTION

This appendix describes in detail the derivation of the procedure used in Chapter 4 to estimate No-Observed-Adverse-Effect level human equivalent concentrations ($NOAEL_{HEC}$s) for extrarespiratory effects of gases or vapors. The derivation is mathematical in nature in that the equations of state that describe the disposition of inhaled compounds in a generalized physiologically based pharmacokinetic (PB-PK) model are manipulated so as to obtain a conservative estimate of $NOAEL_{HEC}$s as a function of the average animal exposure concentrations ($NOAEL_{ADJ}$). A PB-PK model is used because of the success of this type of model. For example, PB-PK models that describe the body as five compartments (gas exchange and the fat, poorly-perfused, richly-perfused, and liver/metabolizing tissue groups) have been applied successfully to estimating the internal concentrations of chemicals (e.g., styrene, methanol, and ethylene dichloride) for the purpose of risk assessment. Although, PB-PK modeling is the choice procedure in risk assessment for dose extrapolation, this approach is not possible without the values of physiological and biochemical parameters, which are used in the modeling process, and without a better understanding of the agent's mechanism of action. These data generally are not available for most compounds.

The proposed method is based on a PB-PK model in which all of any number of compartments are in parallel and in which for any compartment there can be any number of paths of removal by linear and saturable processes. Selected relevant parameter values are replaced by qualitative assumptions about species similarity and the response of internal concentrations to exposure scenarios. In order to obtain a $NOAEL_{HEC}$, the assumption is made that the effective dose for dose-response purposes is the arterial blood concentration of the gas or it's concentration multiplied by time (C x T). (These assumptions are specified in detail in the METHODS section.) This latter assumption is consistent with our current understanding of systemic toxicity for a majority of chemicals, since the toxicity of most environmental chemicals is related to the concentration of the parent compound at the target site over a period of time.

In addition to deriving conservative $NOAEL_{HEC}$ estimates based on arterial blood concentrations, the method also predicts that the blood concentration of an inhaled compound in any human tissue compartment does not exceed the blood concentration in the corresponding animal compartment. Although the present approach does not directly address the issue of metabolites being the toxic agent, the procedure predicts (based on the similarity assumption) that the rate of metabolite production per cardiac output rate or per target tissue perfusion rate in humans does not exceed that in animals.

METHODS

Assumption imposed by the RfD_i methodology:

Assumption I. Noncancer toxic effects observed in chronic animal bioassays are the basis for the determination of NOAELs and the operational derivation of RfD_is for human exposures, as described in Chapter 4. The animal exposure scenario is experiment-dependent and usually intermittent (e.g., 6 h/day, 5 days/week for many weeks). Human exposure concentration is continuous and constant for 70 years. The "lifetime" chronic animal exposure scenario is equivalent to the human chronic exposure scenario for the purpose of extrapolating the NOAEL.

Additional assumptions for the proposed method:

Assumption II. Relatively soon after the beginning of the experiment, and for most of the experiment, all the concentrations of the inhaled gas within the animal's body are periodic with respect to time. Practically, these conditions should be met during "most" of the experiment duration. For example, if the condition is met for nine-tenths of the time (e.g., periodic during the last 90 weeks of a 100-week experiment), then estimates of average concentrations will be in error by less than 10%. During most of the time humans are exposed, given Assumption I of continuous exposure, their internal concentrations are constant and in dynamic equilibrium with their exposure concentration.

Assumption III. A PB-PK model describes the uptake and disposition of inhaled compounds in animals and humans. The model is diagramed in Figure I-1 and the equations of state are given by Equations (I-1) to (I-6). Table I-1 defines the variables and constants in the equations.

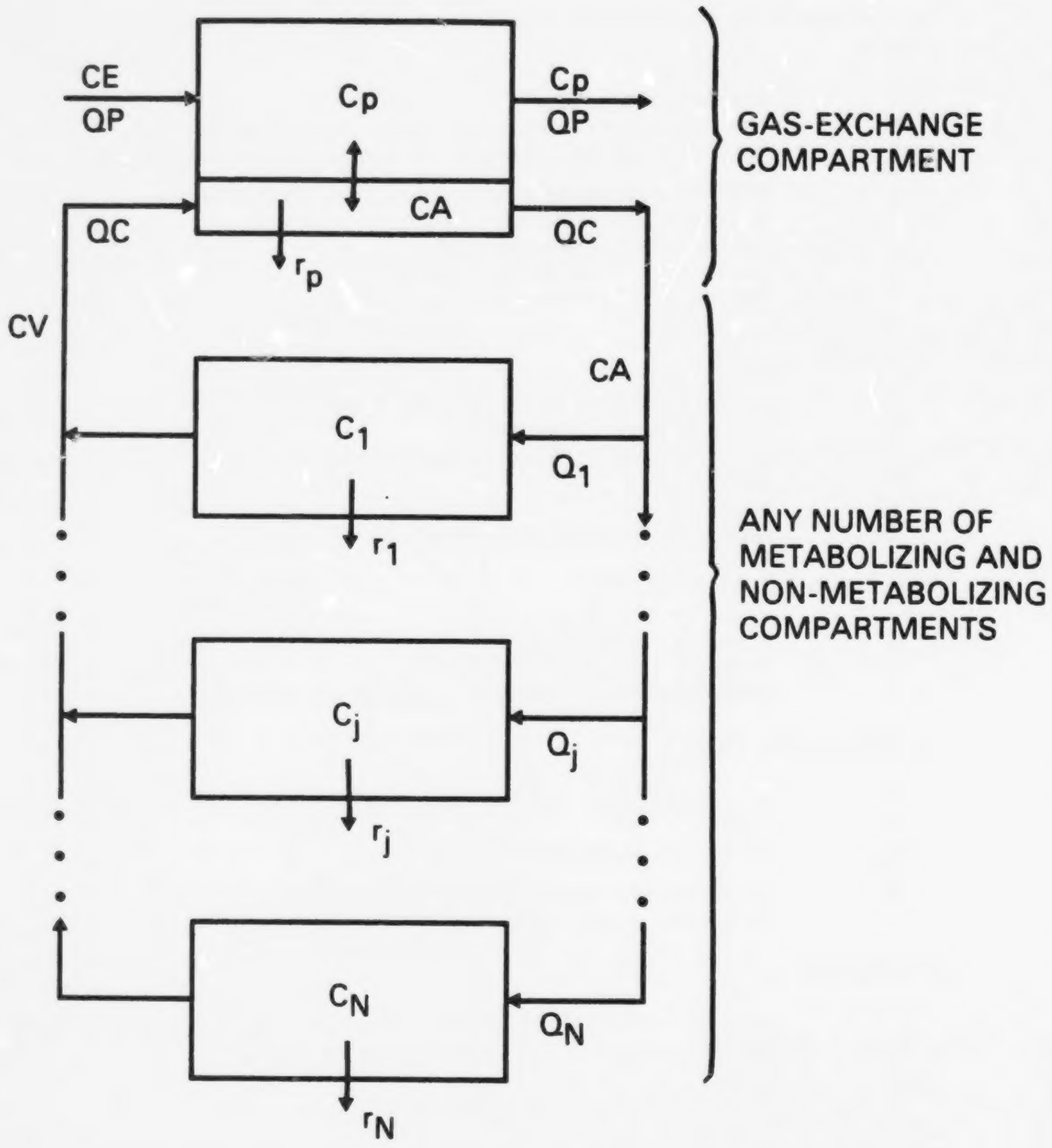

CE
QP
Cp
Cp
QP
CA
QC
QC
rp
CV
CA
C1
Q1
r1
Cj
Qj
rj
CN
QN
rN
GAS-EXCHANGE COMPARTMENT
ANY NUMBER OF METABOLIZING AND NON-METABOLIZING COMPARTMENTS

Figure I-1. Schematic of the physiologically based pharmacokinetic model assumed to describe the uptake and distribution of inhaled compounds.

TABLE I-1. DEFINITION OF SYMBOLS

General	
V	Compartment volume
N	The number of non-gas exchange compartments
M_p	Mass of inhaled compound in gas exchange compartment
M	Mass in compartment other than gas exchange
*	Multiplication symbol
—	Overbar indicates average
λ	Blood to air partition coefficient
T	Period of exposure time
Subscripts	
i	i-th path of loss of primary compound
p	Gas exchange compartment
j	j-th non-gas exchange compartment
A	Animal
H	Human
HEC	Human equivalent concentration
Flow Rates (ml/h)	
QP	Alveolar ventilation
QC	Cardiac output
Q	Into and out of non-gas exchange compartment
Concentrations (mg/l)	
C	In venous blood within and leaving a non-gas exchange compartment
CE	Exposure
C_p	In air of pulmonary region
CA	In arterial blood
CV	In venous blood entering gas exchange region
Biochemical	
r	Removal rate due to metabolism, reactions, excretion, etc. (mg/h)
VMAX	Maximum velocity of saturable path (mg/h)
KM	Michaelis constant (mg/l)
KF	First-order rate constant (1/h)
VKF	Equals to V x KF (1/h)

$$dM_p/dt = QP*(CE - C_p) + QC*(CV - CA) - r_p(CA) \tag{I-1}$$

$$dM_j/dt = Q_j*(CA - C_j) - r_j(C_j);\ j = 1, 2, 3, \ldots N \tag{I-2}$$

$$r_p(CA) = \sum_i VKF_{pi}*CA + \sum_i VMAX_{pi}*CA/(KM_{pi} + CA) \tag{I-3a}$$

$$r_j(C_j) = \sum_i VKF_{ji}*C_j + \sum_i VMAX_{ji}*C_j/(KM_{ji} + C_j);\ j = 1 \text{ to } N \tag{I-3b}$$

$$QC*CV = \sum_j Q_j*C_j \tag{I-4}$$

$$QC = \sum_j Q_j \tag{I-5}$$

$$CA = \lambda*C_p \tag{I-6}$$

Equations (I-1), (I-2), (I-4), and (I-5) are the dynamical equations of state or mass balance equations for the model. Equations (I-3a,b) define the possible loss rates in each compartment in terms of linear rates (e.g., $VKF_{ji}*C_j$) and rates of the Michaelis-Menton type (e.g., $VMAX_{pi}*CA/[KM_{pi} + CA]$). In each compartment, the model allows for more than one path of elimination or metabolism or for no losses (i.e., set all of a compartment's kinetic parameters, VKF and VMAX, to zero). Equation (I-6) gives the assumed relationship between the arterial blood concentration and the concentration in the air of the pulmonary region.

According to Assumption I, the exposure concentration is periodic with period of exposure time (T) for animals and constant for humans; in both cases, concentration of exposure (CE) can be written as:

$$CE = f(t)*\overline{CE} \tag{I-7}$$

where:

$\overline{CE}$ = the average exposure concentration, and

f = a periodic function of time (t) such that:

$$f(t)*dt = 1. \tag{I-8}$$

Assumption IV. Because the biologically effective toxic dose to a given target tissue depends on the animal species and chemical compound, its specification is typically not available so that definition of a surrogate dose must be somewhat arbitrary. However, the toxic effects of some compounds are expected to be directly related to the inhaled parent compound in the blood. Furthermore, the choice of the average blood concentration is conservative and is an internal dose "closer" to the target than a dose based on exposure concentration. Basing the effective dose extrapolation on another surrogate (e.g., metabolite) would require knowledge of the mechanisms of action and additional information about human and animal physiological parameters. Thus, for animal to human exposure extrapolation, the human equivalent exposure concentration (CE_{HEC}) is defined in terms of the time-integrated arterial blood concentration ($CA \times T$) of the inhaled parent compound by requiring that $(CA \times T)_H \leq (CA \times T_A)$. This assumption (combined with Assumption I) is equivalent to requiring that the human equilibrium concentration of arterial blood (leaving the lung) be less than or equal to the time-averaged arterial blood concentration of the animal; that is, $CA_H \leq \overline{CA}_A$. The equality condition defines the upper limit on an acceptable human arterial blood concentration; thus, for mathematical simplicity this assumption is formulated as:

$$CA_H = \overline{CA}_A. \tag{I-9}$$

Because of this requirement, CA_H is a function of $\overline{CE}_A$, since $\overline{CA}_A$ depends on $\overline{CE}_A$.

Assumption V. Similarity of species is assumed in that KM and the ratios Q/QP, VKF/QP, and VMAX/QP are defined as species independent for each removal process (see Table I-1 for definitions). The invariance of the first ratio is based on the assumption that the percent of blood flow to any compartment is independent of species and that cardiac output (QC = sum of all Q_j) scales, with respect to body weight, in the same way as the ventilation rate (QP); i.e., the ratio of QC to QP is species-independent. The metabolic constants VMAX and VKF are assumed to scale in the same way as QP. Justification for this assumption about rates is based on the observation that for many species, rates scale in the same way with respect to body weight; e.g., in proportion to basal metabolism, body surface area, or body weight to some some power (Travis and White, 1988). The invariance of the ratios VKF/QP and VMAX/QP follows.

Subject to the Assumptions, Equations (I-1) to (I-9) must be manipulated to determine CE_{HEC} as a function of the average animal exposure concentration, $\overline{CE}_A$. Because the concentrations and masses of a parent compound within a compartment are assumed to be periodic, the integral of the left-hand side (LHS) of Equations (I-1) and (I-2) over a time length of the period is zero; for example:

$$(dM/dt')*dt' = M(t + T) - M(t) = 0. \qquad \text{(I-10)}$$

Also note that for equilibrium or steady state, as in the human case, the LHS of each of these equations is zero by definition. Performing the period average of both sides of Equations (I-1) to (I-6), the following are obtained.

$$0 = QP*(\overline{CE} - \overline{C_p}) + QC*(\overline{CV} - \overline{CA}) - \bar{r}_p \qquad \text{(I-11)}$$

$$0 = Q_j*(\overline{CA} - \overline{C_j}) - \bar{r}_j; \quad j - 1, 2, 3, \ldots N \qquad \text{(I-12)}$$

$$\bar{r}_p = \sum_i VKF_{pi}*\overline{CA} + \sum_i VMAX_{pi}* \overline{[CA/(KM_{pi} + CA)]} \qquad \text{(I-13a)}$$

$$\bar{r}_j = \sum_i VKF_{ji}*\bar{C}_j + \sum_i VMAX_{ji}*\overline{[C_j/(KM_{ji} + C_j)]}; \quad j = 1 \text{ to } N \qquad \text{(I-13b)}$$

$$QC*\overline{CV} = \sum_j Q_j*\bar{C}_j \qquad \text{(I-14)}$$

$$QC = \sum_j Q_j \qquad \text{(I-15)}$$

$$\overline{CA} = \lambda*\overline{C_p} \qquad \text{(I-16)}$$

The steady state equations for humans are obtained from Equations (I-1) and (I-2) by setting the LHS of these equations to zero (the equilibrium or steady-state condition). The complete set of equations of state for humans can be obtained from Equations (I-11) through (I-16) by redefining the average concentrations or terms as equilibrium values (i.e., remove the overbars).

The above equations are simplified by combining Equations (I-11) and (I-16) to give:

$$(QP/\lambda + QC)*\overline{CA} = QP*\overline{CE} + QC*\overline{CV} - \overline{r}_p, \qquad \text{(I-17)}$$

and Equation (I-12) is expressed as:

$$Q_j*\overline{CA} = Q_j*\overline{C}_j + \overline{r}_j; \quad j = 1 \text{ to } N. \qquad \text{(I-18)}$$

Both sides of Equations (I-17) and (I-18) are divided by QP and Q_j, respectively, to give:

$$u*\overline{CA} = \overline{CE} + w * \overline{CV} - \overline{r}_p/QP, \text{ and} \qquad \text{(I-19a)}$$

$$\overline{CA} = \overline{C}_j + \overline{r}_j/Q_j; \quad j = 1 \text{ to } N \qquad \text{(I-19b)}$$

where:

$$w = QC/QP, \text{ and} \qquad \text{(I-19c)}$$

$$u = (\lambda^{-1} + QC/QP). \qquad \text{(I-19d)}$$

Generally, the constants w and u are species-dependent, and will be identified as such with subscripts A and H for laboratory animal and human, respectively. However, for simplicity and unless otherwise noted, averaged concentrations (indicated by overbar) will be those of animals and nonaveraged concentrations will be those of humans.

Applied to humans, Equations (I-19a) and (I-19b) are written as:

$$u_H* CA = CE + w_H * CV - r_{pH}(CA)/QP_H, \text{ and} \qquad \text{(I-20a)}$$

$$CA = C_j + r_{jH}(C_j)/Q_{jH}; \quad j = 1 \text{ to } N. \qquad \text{(I-20b)}$$

For animals, Equations (I-19a) and (I-19b) are written as:

$$u_A * \overline{CA} = \overline{CE} + w_A * \overline{CV} - \overline{r}_{pA}/QP_A, \text{ and} \qquad \text{(I-20c)}$$

$$\overline{CA} = \overline{C_j} + \overline{r}_{jA}/Q_{jA}; \quad j = 1 \text{ to } N. \qquad \text{(I-20d)}$$

The loss terms in Equations (I-3), $r_p(CA)$ and the $r_j(C_j)$'s, are concave functions with the property that their second derivatives with respect to CA and C_j, respectively, are less than or equal to zero. As a consequence, the average of each of these functions is less than or equal to the function evaluated at the average concentration. Suppressing the subscripts, this property is expressed as:

$$\overline{r} \leq r(\overline{C}). \qquad \text{(I-21)}$$

Considering Equations (I-21), (I-20c), and (I-20d), the following is noted:

$$u_A * \overline{CA} \geq \overline{CE} + w_A * \overline{CV} - r_{pA}(\overline{CA})/QP_A, \text{ and} \qquad \text{(I-22a)}$$

$$\overline{CA} \leq \overline{C}_j + r_{jA}(\overline{C}_j)/Q_{jA}; \quad j = 1 \text{ to } N. \qquad \text{(I-22b)}$$

Using Equation (I-9), Assumption IV (that is, $CA_H = \overline{CA}$, Equations (I-20a) and (I-20b) for human are written in terms of the animal arterial blood concentration by replacing CA with $\overline{CA}$ as follows:

$$u_H * \overline{CA} = CE + w_H * CV - r_{pH}(\overline{CA})/QP_H; \qquad \text{(I-23a)}$$

$$\overline{CA} = C_j + r_{jH}(C_j)/Q_{jH}; \quad j = 1 \text{ to } N. \qquad \text{(I-23b)}$$

Subtract the LHS and the right hand side (RHS) of Equation (I-23a) from the LHS and RHS of Equation (I-22a), respectively, to obtain:

$$(u_A - u_H)*\overline{CA} \geq \overline{CE} - CE + (w_A * \overline{CV} - w_H * CV) - (r_{pA}(\overline{CA})/QP_A - r_{pH}(\overline{CA})/QP_H). \qquad \text{(I-24)}$$

Because of Assumption V, for any concentration value, C:

$$r_{pA}(C)/QP_A = r_{pH}(C)/QP_H, \text{ and} \qquad \text{(I-25a)}$$

$$r_{jA}(C)/Q_{jA} = r_{jH}(C)/Q_{jH}; \qquad \text{(I-25b)}$$

also,

$$w_A = w_H, \text{ and} \qquad \text{(I-25c)}$$

$$u_A - u_H = \lambda^{-1}_A - \lambda^{-1}_H. \qquad \text{(I-25d)}$$

Thus, Equation (I-24) can be written as:

$$(\lambda^{-1}_A - \lambda^{-1}_H) * \overline{CA} \geq \overline{CE} - CE + w*(\overline{CV} - CV), \text{ or} \qquad \text{(I-26a)}$$

$$CE \geq \overline{CE} + w*(\overline{CV} - CV) + (\lambda^{-1}_H - \lambda^{-1}_A) * \overline{CA}. \qquad \text{(I-26b)}$$

Comparing Equations (I-22b) and (I-23b), one sees that the blood concentration of the inhaled compound in any human compartment is less than or equal to the average blood concentration in the corresponding animal compartment; that is:

$$C_j \leq \overline{C}_j. \qquad \text{(I-27)}$$

Because of Assumption V ($Q_{jA}/QC_A = Q_{jH}/QC_H$), it follows from Equation (I-14) applied to both humans and animals, and from Equation (I-27), that:

$$CV \leq \overline{CV}. \qquad \text{(I-28)}$$

Thus, the term $w * (\overline{CV} - CV) \geq 0$ can be dropped from Equation (I-26b) without affecting the inequality as follows:

$$CE \geq \overline{CE} + (\lambda_H^{-1} - \lambda_A^{-1}) * \overline{CA}. \qquad \text{(I-29)}$$

Note that CE is the constant inhaled human concentration that would give rise to a human constant blood level that is no greater than $\overline{CA}$. If we choose the actual human exposure concentration to be less than or equal to the CE, defined by $CA = \overline{CA}$, then the actual CA will be less than or equal to $\overline{CA}$.

The following two cases are now considered with respect to the partition coefficient.

Case I: $\lambda_A \geq \lambda_H$.

The second term on the RHS of Equation (I-29) is greater than or equal to zero; thus, the term can be dropped from the RHS without affecting the inequality. Obviously, a conservative human exposure concentration is $\overline{CE}$. Therefore, in terms of the variables in Chapter 4, a conservative $NOAEL_{HEC}$ is given by:

$$NOAEL_{HEC} = \overline{CE} = NOAEL_{ADJ} \qquad \text{(I-30)}$$

where:

$NOAEL_{ADJ}$ = the observed NOAEL concentration adjusted for exposure duration (Equation 4-3).

Case II: $\lambda_A \leq \lambda_H$.

The second term on the RHS of Equation (I-29) is negative in this instance. The quantity of chemical inhaled must be greater than or equal to the quantity exhaled; this requires that $\overline{CE} \geq \overline{C_p}$ or $\overline{CA} \leq \lambda_A * \overline{CE}$. In Equation (I-29), $\overline{CA}$ can be replaced by the larger value, $\lambda_A * \overline{CE}$, and still preserve the inequality, hence:

$$CE \geq \overline{CE} + (\lambda_H^{-1} - \lambda_A^{-1}) * \lambda_A * \overline{CE}, \text{ or} \qquad \text{(I-31a)}$$

$$CE \geq \overline{CE} *(\lambda_A/\lambda_H). \qquad \text{(I-31b)}$$

In this case, a conservative $NOAEL_{HEC}$ is given by:

$$NOAEL_{HEC} = (\lambda_A/\lambda_H) * \overline{CE} = (\lambda_A/\lambda_H) * NOAEL_{ADJ} \qquad \text{(I-32)}$$

where:

$NOAEL_{ADJ}$ = the observed NOAEL concentration adjusted for exposure duration (Equation 4-3).

RESULTS

A perspective on the proposed method can be attained by examination of Figures I-2 and I-3, plots of $NOAEL_{HEC}$ vs. $NOAEL_A$ for the rat and mouse, respectively. These plots were created by choosing the equivalent exposure concentration that resulted in the human arterial blood concentration being equal to the average arterial blood concentration of the animal, using several methods, for the representative volatile organic compound dicholoromethane (DCM).

The "established" method refers to using ratio of the ventilation rate divided by body weight in the animal to the ventilation rate divided by body weight in the human ratio for calculating $NOAEL_{HEC}$ estimates (Federal Register, 1980), with the modification that alveolar ventilation rates are used (U.S. Environmental Protection Agency, 1988b). The $NOAEL_{ADJ}$ of the animal (Equation 4-3) is multiplied by the ratio to calculate the $NOAEL_{HEC}$ estimate using this method. The "optimal" method refers to the use of the model with an extensive set of experimentally determined physiological parameters for the three species (Andersen et al., 1987). The same model and human parameters were used for the "similar" method, but the animal parameters were determined by scaling from the human values, as defined in Assumption V.

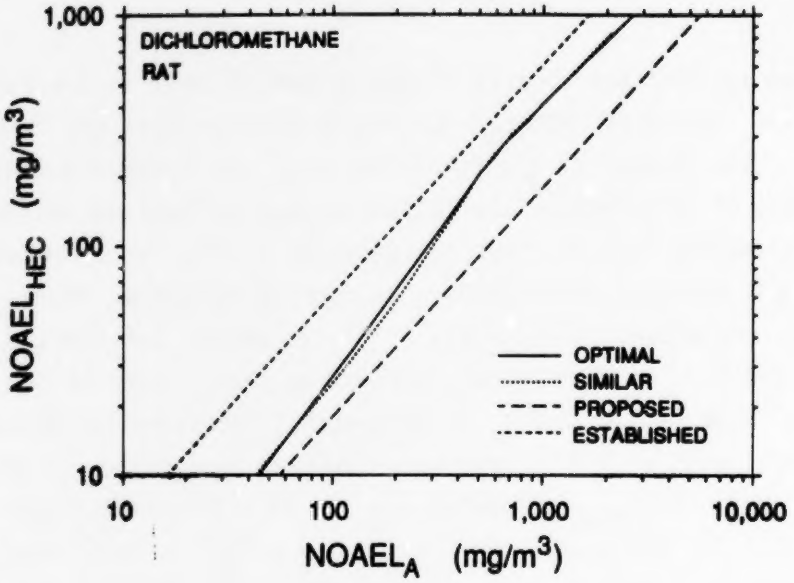


Figure I-2. Plot of NOAEL$_{HEC}$ vs. NOAEL$_A$ for the rat for four possible methods (proposed, established, similar and optimal) of determining NOAEL$_{HEC}$ estimates as defined in the text. The inhaled compound is dichloromethane.

Source: Overton and Jarabek, 1989.

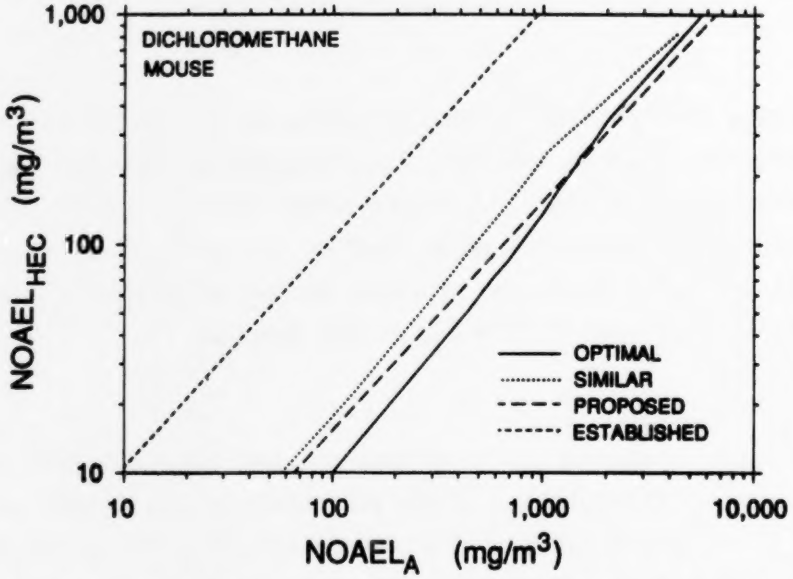


Figure I-3. Plot of NOAEL$_{HEC}$ vs. NOAEL$_A$ for the mouse for four possible methods (proposed, established, similar and optimal) of determining NOAEL$_{HEC}$ estimates as defined in the text. The inhaled compound is dichloromethane.

Source: Overton and Jarabek, 1989.

In keeping with the results of the derivation that is the subject of this Appendix, the "proposed" $\mathrm{NOAEL}_{\mathrm{HEC}}$ estimates are less than the "similar" method estimates. With respect to the relationship of the proposed predictions to the other methods of calculation, the following observations are noted.

The "proposed" method lines are parallel to the "established" lines and result in 3.4 and 6.9 times smaller, or more conservative, $\mathrm{NOAEL}_{\mathrm{HEC}}$ estimates for the rat and mouse, respectively. The "proposed" rat $\mathrm{NOAEL}_{\mathrm{HEC}}$ estimates also fall below (i.e., are more conservative than) those of the "optimal" method by a range of 1.4 to 2.4. Except at high exposure concentrations (above approximately 1,600 $\mathrm{mg/m^3}$), where the estimates are smaller by about 1.3, the "proposed" mouse $\mathrm{NOAEL}_{\mathrm{HEC}}$ estimates are up to 1.5 times greater than the "optimal" $\mathrm{NOAEL}_{\mathrm{HEC}}$ estimates. This supports current evidence that the mouse is not "similar" to humans in some cases (Reitz et al., 1988). The "proposed" method estimates, however, more closely approximate the "optimal" method estimates than do the "established" estimates. It also should be noted that the "optimal", "similar", and "proposed" methods result in more conservative estimates for the mouse vs. rat, whereas the established methodology results in the opposite relationship of estimates between the two species.

DISCUSSION

Considering the "optimal" method estimates to represent the best possible dose extrapolation based on internal blood concentrations, then the "proposed" method is more realistic than the "established" method. Since the blood to air partition coefficients are more readily available than are complete physiological parameter data, the proposed method represents a simple default approach when extensive PB-PK modeling is not feasible.

RESEARCH AND DEVELOPMENT

The approach presented in this Appendix has resulted from modeling research focused on determining the key parameters of gas uptake, distribution and target tissue accumulation. The future effort will incorporate the anatomic and some aspects of the clearance data being compiled for research to support the particle modeling as described in Appendix H. Model evaluation plans include comparing the efficiency of various dose surrogates and an approach to address the apparent non-similarity of the mouse. Application of the model to address mixtures of gases and of dose partitioning between gas and particles is also envisioned.

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